Universidade de Lisboa

Faculdade de Farmácia

Evaluation of the antifungal activity of chitin binding lectins from *Mauritia flexuosa* and *Artocarpus incisa* in *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*

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Dissertação orientada pela Professora Doutora Ana Cristina de Oliveira Monteiro Moreira e coorientada pela Professora Doutora Olga Maria Duarte Silva

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RESUMO

Fungos são seres eucarióticos, protistas, não fotossintéticos, sua estrutura celular tem grande similaridade com as células humanas, diferenciando se apenas por conter quitina (que é um polímero de cadeia longa de N-acetilglucosamina) em sua parede celular, esta similaridade dificulta a ação de medicamentos anti fúngicos convencionais pois estes não são tão seletivos, o que pode causar efeitos adversos e resistência do patógeno aos medicamentos. Normalmente indivíduos saudáveis conseguem resistir a infecções fúngicas, porém em grupos de pacientes com sistema imune prejudicado por medicamentos anticancerosos ou portadores de condições específicas como AIDS ficam sujeitos a infecções fúngicas oportunistas principalmente por *Cryptococcus neoformans, Candida albicans e Aspergillus fumigatus.*

Uma alternativa terapêutica seria buscar novos compostos de preferência com baixo custo e alvo molecular mais seletivo para células fúngicas com menor citotoxicidade em células humanas, como por exemplo, as lectinas que são proteínas de origem não imunológica encontradas em microorganismos, animais e mais frequentemente em vegetais, que são capazes de se ligar reversivelmente e seletivamente à hidratos de carbono específicos sem alterar sua estrutura.

Estas proteínas são de grande interesse científico devido às outras propriedades imunológicas (ativa sistema complemento fagocitando patógenos), farmacológicas (medicamentos de liberação prolongada, biomarcadores); antitumoral (reconhece hidratos de carbono específicos de células neoplásicas); anti-HIV1 (inibe proteases e transcriptase reversa) e antimicrobianas (aglutina fungos e bactérias), o que as torna uma importante

ferramenta biotecnológica na identificação de receptores de membrana e estudos citoquímicos e farmacológicos.

As lectinas podem ser classificadas de acordo com sua estrutura e evolução (quitina-ligantes contendo domínios de heveína, inativadoras de ribossomos, manose-ligantes de monocotiledôneas e lectinas relacionadas à jacalina) ou classificadas pela especificidade de ligação a hidratos de carbono específicos (ligantes de manose, glicose, maltose, N-acetilglicosamina) e finalmente classificadas de acordo com sua estrutura global (Merolectinas que se liga a apenas um hidrato de carbono, Hololectinas que possuem dois ou mais sítios ligantes a hidratos de carbono idênticos, Quimerolectinas que possuem pelo menos um sítio ligante a hidratos de carbono e outro sítio com outra atividade biológica, Superlectinas que possuem dois ou mais domínios ligantes a hidratos de carbono mas diferentes das hololectinas reconhecem hidratos de carbono com estruturas diferente e as Multilectinas que possuem dois ou mais domínios ligantes a hidratos de carbono, idênticos, mas que podem se ligar a açúcares diferentes.

A purificação de lectinas é feita principalmente através de cromatografia de afinidade, onde o extrato bruto a conter lectinas passa através de uma matriz com o hidrato de carbono que a lectina em estudo tem afinidade, e as outras moléculas sem afinidade passam sem se ligar, enquanto que as moléculas específicas para o extrato, no caso hidratos de carbono , são retidas e a proteína desejada é obtida com alto grau de pureza e seletividade, posteriormente será libertada da coluna e dialisada, obtendo se um extrato proteico puro e concentrado.

Artocarpus incisa possui lectinas muito semelhantes às lectinas de *Artocarpus integrifólia,* que já são bem caracterizadas, descobriu-se uma lectina inédita em *A.incisa* chamada de Frutaquina que interagiu com a matriz de quitina em cromatografia de afinidade e apresenta similaridade estrutural com Jaquina que é uma lectina ligante de

quitina de *A.integrifolia*, o que estimulou avaliar sua purificação, rendimento e actividade anti fúngica.

As monocotiledôneas como *Mauritia flexuosa* L.f. são pouco estudadas e carecem de mais estudos de investigação de suas lectinas, normalmente possuem lectinas termoestáveis o que garante a estabilidade de sua atividade biológica e também possuem bom rendimento de extração, estudos anteriores demonstraram que o extracto proteico de *M. flexuosa* interagiu com a matriz de quitina, comprovando que ela pode ser uma possível fonte de lectinas quitina ligante.

Mauritia flexuosa L. f. e *Artocarpus incisa* são plantas que possuem lectinas que se ligam seletivamente a quitina presente na parede celular de fungos e podem servir como alternativa terapêutica para combater fungos patogênicos oportunistas, então o objetivo deste trabalho é obter um extracto proteico puro e concentrado destas lectinas e avaliar principalmente sua atividade antifúngica em três fungos patogênicos oportunistas que são *Cryptococcus neoformans, Candida albicans e Aspergillus fumigatus.*

Para purificação das lectinas de *M. flexuosa* utilizou-se o tampão Citrato Fosfato 0,1M pH 6,5 com NaCl 0,15M. O extrato foi submetido a fracionamento com Sulfato de Amônio 60% (F60) e foi utilizado na cromatografia de afinidade em Quitina equilibrada com Tampão Citrato Fosfato 0,01M pH 6,5 com NaCl 0,15M e depois dialisado exaustivamente com água de osmose overnight. O Extrato proteico foi então submetido a ensaios de Termoestabilidade, Aglutinação em *Saccharomyces cerevisiae*, e ensaios anti fúngicos.

Na purificação da lectina ligante de quitina de *A.incisa chamada* Frutaquina, o extrato bruto foi submetido a purificação de outras lectinas presentes na amostra para posteriormente isolar a lectina ligante de quitina, então primeiro ele foi aplicado em coluna de galactomanana para reter lectinas D-galactose ligantes (Frutalina), depois a fração não ligada anteriormente foi aplicada em coluna de sepharose-D-manose para retirar as lectinas D-manose ligantes (Frutapina) e a fração não ligada foi aplicada em coluna de quitina equilibrada com tampão PBS e eluída onde se obteu o extrato proteico de Frutaquina pura. Como o rendimento de purificação da Frutaquina foi baixo (3 mg) só foi possível realizar a Eletroforese e o ensaio de aglutinação em *Saccharomyces cerevisiae*, não sendo possível realizar os ensaios anti fúngicos, pois iria demandar muito tempo, visto que seu processo de purificação é bastante demorado e complexo e a estabilidade do extrato proteico poderia ser comprometida. Frutaquina foi capaz de aglutinar *Saccharomyces cerevisiae* na concentração de 1mg/mL e a sua Eletroforese mostrou que ela possui perfil eletroforético muito similar a Jaquina, que é outra lectina ligante de quitina que possui similaridade aminoacídica de 98% com Frutaquina, desta forma é possível que ambas estas lectinas possuam atividades semelhantes.

As lectinas de *M. flexuosa* possuem um rendimento bem mais satisfatório do que as de *A.incisa*, onde 50g de sementes de *M. flexuosa* se obteu 30 mg de proteína pura, apresentou alta resistência a variação de temperatura, onde mesmo após ser autoclavada a 100 °C por 20 minutos ainda manteve sua actividade de ligar-se seletivamente a matriz de quitina e aglutinar *S. cerevisiae*. Nos ensaios anti fúngicos, as lectinas quitina ligantes de *M. flexuosa* foram capazes de aglutinar *Cryptococcus neoformans* e *Candida albicans* na concentração de 5 mg/mL e associado à Fluconazol 5 mg/mL de Fluconazol + 5 mg/mL de extrato proteico com lectinas), porém não foi capaz de inibir o crescimento de *Aspergillus fumigatus.*

Portanto, as lectinas ligantes de quitina são uma alternativa terapêutica promissora para investigação e desenvolvimento de novos biofármacos com alvo molecular mais seletivo e menor possibilidade de efeitos colaterais. A Frutaquina purificada de *A.incisa* possui rendimento muito baixo, porém exibe potencial anti fúngico, sugere-se a possibilidade de estudos de investigação para utilizar a tecnologia do DNA recombinante para produção de proteínas recombinantes em grande escala. As lectinas purificadas de *M. flexuosa* são uma opção mais acessível e de baixo custo, com bom rendimento, grande resistência a altas temperaturas e potencial anti fúngico satisfatório, além de não aglutinar eritrócitos humanos, desta forma, provavelmente apresenta segurança para ser incorporada em formas farmacêuticas de uso tópico ou oral.

Estudos posteriores são necessários para comprovar a segurança destas lectinas e avaliar qual a melhor forma farmacêutica para incorporação além de outras possíveis atividades biológicas, para produção de novos fármacos de uso oral pode-se aplicar nanopartículas de galactomananas e xiloglucanas para medicamentos de liberação controlada para garantir melhora da farmacocinética e farmacodinâmica e para desenvolvimento de pomadas pode se aplicar géis à base de hemiceluloses vegetais contendo lectinas D-galactose ligantes para modulação do processo cicatricial e melhora das lesões causadas pelos fungos associada ao poder aglutinante das lectinas ligantes de quitina.

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"Se você consegue sonhar algo, consegue realizá-lo!"

Walt Disney

"A nossa maior glória não reside no fato de nunca cairmos,

e sim em levantarmo-nos sempre depois de cada queda."

Oliver Goldsmith

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LISTA DE ABREVIATURAS:

- ADS: Sabouraud dextrose agar
- AIDS: Acquired Immunodeficiency Syndrome
- AOX :alternative ubiquinol oxidase
- CRC: the classical respiratory chain
- DNA: Deoxyribonucleic Acid
- F60: Fractionation with Ammonium Sulfate 60
- F630: Fractionation with Ammonium Sulfate 30
- GP120 : Glycoprotein 120
- GP41 : Glycoprotein 41
- HIV 1: Human Immunodeficiency Vírus type 1
- HPLC – Cromatografia líquida de alto desempenho (High Performance Liquid Chromatography)
- HPLC-RP : Reversed Phase High performance Liquid Chromatography
- IgE: Immunoglobulin E
- MBL : Mannose-Binding Lectin
- PAR :parallel respiratory chain
- PBS: Phosphate buffered saline

- RNA: Ribonucleic Acid
- SDS-PAGE : Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

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1 - INTRODUCTION

The evolution of the physiological adaptations developed by plants as a defense mechanism against herbivores and microorganisms includes, for example, thorns, trichomes, hard stems and thicker or smoother ,foliar surfaces as well as chemical defense mechanisms that can be mediated by various substances, including primary metabolites (defense proteins) and secondary (alkaloids, flavonoids, saponins, tannins, terpenes) that act in different ways causing toxicity to the predator. A long time ago humans knew that some tubers, grains of cereals and legumes could not be eaten raw because they caused damage to the body, but the specific cause of its toxicity was not known. Today it is known that many of these vegetables have lectins, which are phytochemicals that act as a defense mechanism against attacks by microorganisms (bacteria, viruses, fungi and parasites), insects and other herbivorous animals. Many lectins are toxic to our body, even when subjected to high temperatures in cooking, some lectins decrease their toxicity, however most are very active biochemically, still maintains their biological activity even after high temperatures. (Sharon & Lis, 2002 ; Van Damme *et al.,* 1995 ; Moreira *et al.,* 1990; Goldstein *et al.,* 2007).

The name lectin, originated from the Latin *"lectus",* meaning selected, originally refers to the ability of this specific class of proteins of non-immunological origin to selectively and reversibly bind to carbohydrates, it is important to focus the non-immunological origin of the lectins, since serves to distinguish them from anti-carbohydrate antibodies that agglutinate cells, where as antibodies are structurally similar, lectins differ among them in amino acid composition, metal affinity, molecular weight and three-dimensional structure (Sharon & Lis, 2002; Van Damme *et al.* , 1995; Moreira *et al.,* 1990).

Lectins are currently defined as proteins of non-immune origin that bind hydrophobically (hydrophobicity is the main interaction force between lectins and carbohydrates through hydrophobic sites) in a reversible manner to carbohydrates, as they are glycoproteins, they have the ability to bind cells and precipitating glycoconjugates due to their specific recognition and binding ability without, however, altering the structure of any glycosyl are present not only in plants but also in microorganisms and animals, and those found in higher plants have been studied more by their actions fungicide, antimicrobial and insecticide, in addition to stimulating cells of the immune system (Freire, 2003 ; Van Damme *et al.,* 1995; Moreira *et al.,* 1990; Kennedy *et al.,* 1995).

The first studies on lectins began after Stilmark in 1888 at the University of Dorpad, isolated lectins from *Ricinus communis L*., and observed its ability to agglutinate erythrocytes. Initially it was thought that hemagglutination was some bacteria, later it was confirmed that the material responsible for hemagglutination was a lectin class protein, which was later called “Ricin” (Stilmark 1888 ; Kennedy *et al.,* 1995; Van Damme *et al.,* 1995; Monteiro Moreira 2002).

Lectinology is the science that investigates and characterizes lectins, its property of selectively binding to carbohydrates constitutes an important study tool in the biotechnological, glycobiological and glycobioquimic areas, such as, for example, structural and functional investigation of complex carbohydrates, changes In the cell surface binding activity among other applications, the main source producing lectins are plants, so most of the lectins studied are from vegetable origin (Wu *et al.,* 2006).

2 - TOXICITY OF LECTINS

The binding ability of the lectins allows them to bind to villi of the small intestine causing intestinal damage such as impaired cell repair, cell death, reduced absorption of other nutrients (including minerals and other proteins), and also alters the intestinal flora allowing the growth of *Escherichia coli.* Examples of plants containing toxic lectins are grains (wheat, wheat germ, quinoa, rice, oats, rye, barley, corn), beans (including soybeans and peanuts) and solanaceas (vegetables grown at night, such as Potatoes, tomatoes, eggplants and peppers), in order to avoid the high toxicity of lectins, these vegetables should only be consumed when well matured and after heating (Peumans *et al.,* 2003; Matser *et al.,* 2005, Douglas *et al.,* 1999).

Examples of lectins with cytotoxic action for animals cells are ricin, abrin and, to a lesser extent, *Canavalia ensiform* agglutinin (Concanavalin A - Con A) and WGA (wheat), the toxic action of lectins on cells is generally selective , are more active on transformed cells, which are more sensitive to their effects, when compared to normal cells. The lectin of *Phaseolus acutifolius* was tested in vivo in mice and showed to have low toxicity in normal cells, this proves their selectivity in transformed or altered cells, and can be used in carcinogenic studies that can optimize cancer therapy. Jacalin and Artocarpin are mitogenic to lymphocytes, stimulate the production of endogenous cytokines and the attraction and activation of leukocytes. WGA is a non-mitogenic lectin, however, modulates the defense response by stimulating the release of superoxide by neutrophils. The understanding of toxicity and other biological activities will allow the use of these molecules as a useful tool in the diagnosis and treatment of many diseases. (Reynoso-Camacho *et al.,* 2003).

3 - OCCURRENCE AND FUNCTIONS OF LECTINES

The lectins are found in unicellular organisms (Imbert *et al.,* 2004), animals (Moura *et al.,* 2006) and vegetables (Leite *et al.,* 2005), the most studied are plant lectins because they are found in large quantities and are more abundant in the purification process (Wu *et al.,* 2006), they are isolated from storage organs (mainly seeds with larger amounts but also tubers, (Wang *et al.,* 2005), roots (Wang & Ng, 2006), and other plant tissues, such as leaves and Flowers (Suseelan *et al.,* 2002; Suseelan *et al.,* 2007).

3.1 Lectins in Fungi and Bacteria

Among the microorganisms, fungi have great importance in the research involving the isolation and characterization of new lectins, since they are a great biological source such as, for example, mushrooms and yeasts, bacteria and cyanobacteria have been evaluated for the detection and obtaining of lectins, but with fewer studies (Li *.,* 2007; Wälti *et al.,* 2008; Goldstein *et al.,* 2007; Jung *et al.,* 2007; Thakur *et al.,* 2007; Syed *et al.,* 1999; Yamaguchi *et al.,* 1999; Yamaguchi *et al.,* 1998).

3.2 Lectins in Animals

The lectins are also present in the body of animals and occur in representatives of almost all phyla, in invertebrate animals we have studies of protozoa (Babal ; Russel, 1999), insects (Ourth *et al.,* 2005), molluscs (Takahashi *et al.,* Moura *et al.,* 2006), and sponges (Moura *et al.,* 2006). In vertebrate animals, lectins have been isolated and characterized from amphibians (Lerivray *et al.,* 2001), from fishes (Bazil & Entlicher, 1999;) and other organisms. In man in particular, there are now several well-characterized lectins present in different tissues and cells of the body, such as in the lung (Sorensen *et al.,* 2007, Kishore *et al.,* 2006, Dunphy *et al,.* 2002 Kanazawa *et al.,* 2004), in the serum (Wallace, 2007, Bouwman *et al.,* 2006), in placenta (Soilleux and

Coleman, 2003), among others. Plays important role in the complement system in the pathway of the lectins where mannose-binding lectin (MBL) binds to mannose, fucose or glucose residues and other sugars, which cover the cell surface of many pathogens and activates the cascade of the complement system, this pathway participates in the innate immune response, since it is not mediated by antibodies, MBL deficient individuals are more susceptible to infections in childhood, which shows the importance of the lectin pathway in host defense (Turner, 2003)

3.3 Lectins in Plants

Lectins are normally detected, isolated and purified from plants, they are a great source of lectins that are used for analysis and isolation in seeds (vegetable organ with higher lectin content, corresponding to 10% of the total protein present in this tissue) of the family *Leguminosae* (Spilatro *et al.,* 1996; Sharon & Lis, 2002).

They have several physiological functions in the plant, such as nitrogen reserve, defense against viruses and microorganisms, action against insects, regulation and cellular signaling, against herbivorous predator animals (interacting with their glycoconjugates present in these animals adhering to the gastrointestinal tract causing gastrotoxicity) , plant cell wall growth and carbohydrate transport (Ripoll *et al.,* 2003; Wang, Ng 2003, Bandyopadhyay *et al.,* 2001, Peumans, Van Damme 1995).

Most of the lectin research studies purify more seed lectins (Singh *et al.,* 2007; Sitohy *et al.,* 2007; Sono *et al.,* 2007; Konozy *et al.,* 2003) because they are large sources of lectins, but they are also detected in other tissues or plant organs, less frequently and in smaller quantities, such as in tree barks (Wititsuwannakul *et al.,* 1998), in tubers (Kaur *et al.,* 2006; Kaur *et al.,* 2005), bulbs (Bertrand et al., 1998; Parisi et al., 2008) leaves (Rameshwaram; Nadimpalli, 2008; Ooi *et al.,* 2004) fruits (Wang; Ng, 2006; Peumans *et*

al., 1998), roots (Naeem *et al.,* 2001), rhizome (Chu; Ng, 2006; Kaur et al., 2005), cotyledons (Gupta; Srivastava, 1998), in the latex of some species (Seshagirirao; Prasad, 1995;) and other parts of the plants, in addition there are studies on algae (Leite *et al.,* 2005; Ambrosio *et al.,* 2003) and, to a lesser extent, lichens (Elifio *et al.,* 2000; Molina; Vicente, 2000).

However, many lectins from other families have also been frequently isolated and characterized as, for example, *Solanaceae* lectins (Peumans et al., 2003), *Labiateae* (Fernández-Alonso et al. 2003) and *Moraceae* (Moreira *et al.,* 1981), among others, it is important to consider the plant species to be investigated, since it is a decisive factor in the search for unpublished or homologous lectins.

4 - CHEMICAL AND STRUCTURAL CHARACTERISTIC OF LECTINS

The specificity of lectins to carbohydrates occurs through hydrogen bonds, hydrophobic interactions, and Van der Waals forces, hydrophobic regions of these proteins interact with hydroxyl groups in carbohydrates, which because of their spatial arrangement create hydrophobic sites. They are more specific for disaccharides and polysaccharides than monosaccharides, may contain from 2 to 12 interaction sites, depending on the nature of the molecule and its oligomerization state, they exhibit high similarity in their amino acid residues, including those involved in binding to carbohydrates and most of those that coordinate the metal ions necessary for the integrity of the subunits and the correct positioning of amino acid residues for binding, the structural differences between lectins occur from the primary structure to the last degree of molecular organization, they may be different in the amino acid sequence, in the variation of the number of subunits per molecule and in the nature of the polypeptides. (Sharon & Lis, 2002).

Structurally they are divided into four categories, Merolectins which have one binding carbohydrate site, Hololectins with several homologous sites that bind to the same sugar, Chimerolectins have a carbohydrate binding site and another site with another biological activity and Superlectins having carbohydrate domains which recognize different sugars (Spilatro *et al.,* 1996; Peumans , Van Damme *et al.,* 1995; Balzarini, 2006; Sharon & Lis, 2002).

5 – CLASSIFICATION OF LECTINS

In order for a protein to be classified as lectin, it must have some specific characteristics and properties, such as: being a carbohydrate binder (tannins, lipids that also have binding activity are excluded), have no immunological origin (previously they were considered antibodies because of their specificity, but do not need immunological stimulation to be synthesized and activated) and do not biochemically and structurally alter the carbohydrates that will bind (Rudiher & Gabius, 2001)

Lectins are different in relation to their structure, specificity, biochemical and physicochemical properties, in this way they can have several different classifications and can be classified as follows:

5.1 Structural and evolutionary classification:

- Chitin-binding lectins containing hevein domains
- Lectins of *Legumenaceae* (the most studied of them all)
- Inhibitory Ribosome Lectins - RIPs
- Mannose binding lectins of Monocotyledons
- Lectins related to Jacalin

(Garcia-Pino *et al.*, 2006; Stirpe *et al.*, 2007; Pelosi *et al.*, 2005; Hartley; Lord 2004; Barre *et al.*, 2004; Rougé *et al.*, 2003).

5.2 Classification according to Carbohydrate binding specificity:

• Mannose / glucose-binding lectins

• Mannose / maltose binding lectins,

• Galactose / N-acetylgalactosamine-binding lectins

• Lectins binding to N-acetylglucosamine / (N-acetylglucosamine)

(Machuka et al., 2000; Koike et al., 1995; Nomura et al., 1998).

5.3 Classification according to global structure:

- Merolecins: Lectins possessing a single carbohydrate binding domain, are small proteins, formed by a single polypeptide and because of their monovalent nature, are incapable of precipitate glycoconjugates or agglutinate cells, for example, hevein which is a binding protein of chitin Isolated from rubber tree latex (*Hevea brasiliensis*). (Monteiro Moreira 2002, Van Damme et al., 1995; Peumans *et al.,* 1996).
- Hololecins : Lectins having two or more identical or very homologous carbohydrate binding domains, can agglutinate red blood cells and precipitate glycoconjugates, comprise all lectins that have multiple binding sites, such as the jacalin present in *Artocarpus integrifolia*. A large amount of lectins present in plants behaves like hemagglutinins, belonging to this group. (Monteiro Moreira 2002, Van Damme *et al.,* 1995; Peumans *et al.,* 1996).
- Chimerolectins: Fusion of proteins having at least one carbohydrate binding domain, and another domain with a catalytic activity or other biological activity such as ricin from *Ricinus communis L*. and lectins from *Viscum album L*.. May behave like merolectinas or hololectinas depending on the number of carbohydrate

binding sites. (Monteiro Moreira 2002, Van Damme et al., 1995; Peumans et al., 1996).

- Superlectins: have two or more carbohydrate binding domains, but different from hololectins, recognize carbohydrates with different structures, the TGL lectin from *Tulipa gesneriana L.* which has a D-Manose binding domain and another N-acetyl-D-Galactosamine, is an example of this class. (Monteiro Moreira 2002, Van Damme et al., 1995; Peumans et al., 1996).

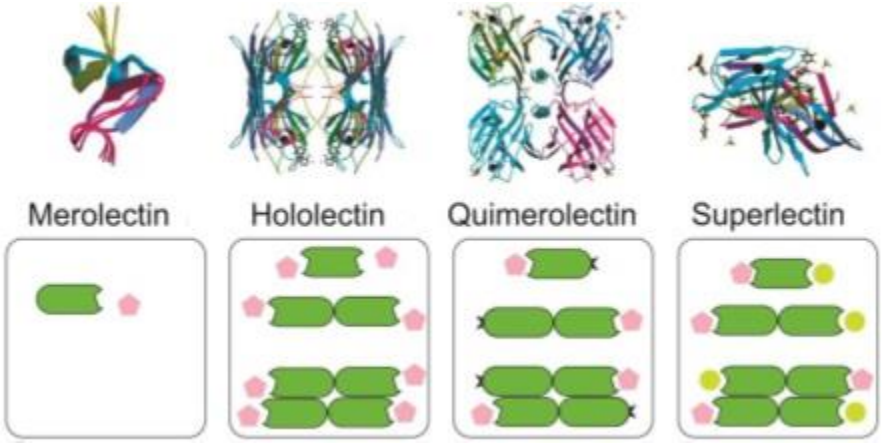


Figure 1: Classification according to their global structure: Merolectins , Hololectins, Chimerolectins, Superlectins and their respective ligands. The lectins are the dark green ligands, the carbohydrates are the pink and light green ligands. The Merolectins bind only to a specific carbohydrate ,Hololectins bind to two similar carbohydrates, Chimerolectins have a carbohydrate binding site and another site with another biological activity and the superlectins are able to bind to different carbohydrates. Source: Adapted from Correia 2015

6 - PURIFICATION OF VEGETABLE LECTINS

The research studies in lectinology are formed by several stages, first we have to choose the source of lectins to obtain the crude extract, these sources can be from animals, microorganisms or plants. In this work we will focus on the vegetable lectins due to being the largest source, especially those of the *Leguminosae* family, the crude extract can be obtained from a saline solution, as in the case of the isolation of lectin from *Erythrina speciosa* and *Morus nigra*, monitoring assays such as agglutination or haemagglutination will be required throughout the extractive process to check whether the sample has binding lectins, then one should choose a method to purify the crude extract, such as chromatography, and then release these purified proteins and dialyse to remove other remaining substances. (Rougé *et al.,* 2003; Konozy *et al.,* 2003; Suseelan *et al.,* 2002; Rojo *et al.,* 2003; Delatorre *et al.,* 2006; Thakur *et al.,* 2007).

6.1 Obtaining the Crude Vegetable Extract

Purification of plant lectins begins with extraction of plant material (leaves, flowers, stems or seeds) using suitable buffers, as they are water-soluble they are extracted with aqueous buffers, then the crude extract is obtained with defined concentration, time and temperature under constant stirring, then it is subsequently filtered to remove impurities and the supernatant is centrifuged at 4°C to avoid denaturation and degradation by proteolytic enzymes, after centrifugation the supernatant is filtered on small pore membrane filters and then the crude extract is obtained . If the plant material has many impurities it will be necessary to carry out a previous purification as in the case of Buriti (*Mauritia flexuosa*) which needs to undergo several processes of delipidation to remove the excess lipids before being extracted with buffer (Kvennefors et al., 2008; Matsumoto et al., 2011; Watanabe et al., 2007).

6.2 Fractionation with Ammonium Sulfate

After the crude extract is obtained further purification is done using saline fractionation based on the separation of molecules according to their solubility differences, the precipitation using ammonium sulfate $(NH_4)_2SO_4$ alters its ionic strength and affects the solubility of the proteins. The “Salting in Effect” is when ammonium sulfate is added in reduced concentrations that decreases the interaction between proteins increasing their solubility in aqueous solution, in the “Salting out Effect” large amounts of ammonium sulfate are added increasing the interaction between proteins reducing the solubility causing precipitation. Different fractions may be proceeded for the partial purification of several lectins in an extract, since different proteins have different reactions in response to salt concentrations. The most commonly used salt is ammonium sulfate because of its high solubility to allow protein precipitation in solutions with high ionic strength and because it does not denature proteins. (Rougé *et al.,* 2003; Konozy *et al.,* 2003; Suseelan *et al.,* 2002; Rojo *et al.,* 2003; Delatorre *et al.,* 2006; Thakur *et al.,* 2007).

6.3 Chromatography:

Chromatography (derived from the Greek words "khromatos" which means color and "graphein" meaning writing) is a physicochemical method for separate the components of a mixture, realized by distributing these components in two phases, one phase is stationary and the other is the mobile, during the passage of the mobile phase by the stationary, the components of the mixture are distributed between two phases, such that each of the components is selectively retained by the stationary phase, resulting in different migrations of the components. There are several chromatographic methods for purification of lectins from the crude extract, which vary according to the matrices that are used, whose choice depends on the specificity to

carbohydrates, liquid charge (ion exchange chromatography) and molecular size of the protein (Gel filtration chromatography) (Sun et al., 2007; Santi-Gadelha ., 2006; Moura et al., 2006, Peumans & Van Damme, 1995).

Ion-exchange chromatography is based on the binding of the protein to the groups of counter signals immobilized on the matrix, molecular exclusion chromatography is based on the separation of biomolecules according to their size and affinity chromatography which is based on selective isolation of biological macromolecules using the ability of proteins to specifically bind to other molecules by affinity, such as lectins, which bind specifically to carbohydrates having affinity through reversible bonds. (Sun et al., 2007; Santi-Gadelha ., 2006; Moura et al., 2006, Peumans & Van Damme, 1995).

Affinity chromatography is ideal for purifying lectins because these ligands are immobilized to the matrix, promoting a selective stationary phase, the crude extract is applied to the matrix and the other molecules without affinity pass without binding, whereas the molecules specific for the extract In the carbohydrate case are retained and the desired protein is obtained with a high degree of purity and selectivity. Various carriers may be used in affinity chromatography, such as cellulose matrix, guar gel, agarose, chitin, sepharose, sephadex. (Pfaunmiller et.al 2013; (Kvennefors et al., 2008; Matsumoto et al., 2011; Watanabe et al., 2007).

In affinity chromatography the protein is separated into a single biochemical property, wherein the linker (molecule that the target protein has affinity) is covalently linked to the matrix (must be porous, chemically inert, have functional groups suitable for coupling with different linkers), there is no single method, it must be individualized according to the type of lectin that is working, one must choose an appropriate lectin

ligand matrix, elution technique more appropriate to the type of protein (Pfaunmiller et.al 2013).

6.4 Elution:

To release lectin from the affinity column, competitive elution or elution with different ionic strength or pH may be applied, some important factors must be considered as specificity, stability (some lectins are sensitive to very acid pH and protein denaturation may occur) and type of adsorbent carbohydrate to be used as a ligand (Cammarata *et al.,* 2007 , Che *et.al* 2011, Rougé *et al.,* 2003, Konozy et al., 2003; Suseelan *et al.,* 2002, Rojo *et al.,* 2003; Delatorre *et al.,* 2006; Thakur *et al.,* 2007).

6.5 Dialysis:

Then the lectins released from the column by elution are generally subjected to a thorough dialysis process on selective membranes, is a method based on the separation of molecules by molecular weight differences, the proteins are retained, whereas smaller molecules such as carbohydrates or salts present in the sample pass into the solvent solution outside the membrane which may be either distilled water or osmosis water (Pfaunmiller et.al 2013).

6.6. Assays after Purification Process:

Lectins have properties like especially to bind to glycoconjugates, are important tools for research in several areas of science, especially biotechnology, these proteins have the most varied effects on cells, among them, agglutination, mitogenic stimulation , redistribution of cell surface components, modification of enzyme activity, inhibition of bacterial and fungal growth, toxicity, immunosuppressive effect, among others, once purified are submitted to Physical-Chemical and Biological tests to evaluate their properties. (Bertrand *et al.,* 1998 ; Takahashi *et al.,* 2008; Sitohy *et al.,* 2007; Kaur *et al.,* 2006).

6.6.1 Biological Assays:

6.6.1.1 Agglutination and Hemagglutination Assays

The agglutination and haemagglutination assay are used for the detection of lectins during and after the purification process to ensure that there is presence of lectins in the crude extract and in the final protein extract, it is useful to characterize them for specificity within the human ABO system (for example, type A blood has N-acetylgalactosamine on the surface of the erythrocyte, blood group B has terminal galactose and group O has fucosyl-galactose) or between erythrocytes of animals, in which the carbohydrates present in the glycocalyx interact reversibly with the lectins, this interaction of the lectins contributes to the choice of the ideal affinity matrix for the purification of the same, most of the lectins agglutinate erythrocytes so the hemagglutination test is usually done, but some specific ones such as the chitin binders prefer to agglutinate fungi (Wälti *et al.,* 2008, Thakur *et al.,* 2007 ; Correia *et al.,* 2008; Van Damme *et al.,* 1995).

The agglutination and hemagglutination assays are performed by serial dilution of the extract containing lectin and subsequent incubation with human or animal erythrocytes or sometimes fungi which promotes the easy visualization of this binding property of human/animals erythrocytes or fungi, the presence of lectins by the hemagglutination assay is confirmed by the phenomenon of inhibition of this hemagglutination in the presence of one (or more) carbohydrate (s) at a concentration determined , which also serves to confirm which carbohydrates the lectin has affinity. (Jung et al., 2007; Goldstein et al., 2007; Santos et.al 2005, Wu, J. H. et al., 2006).

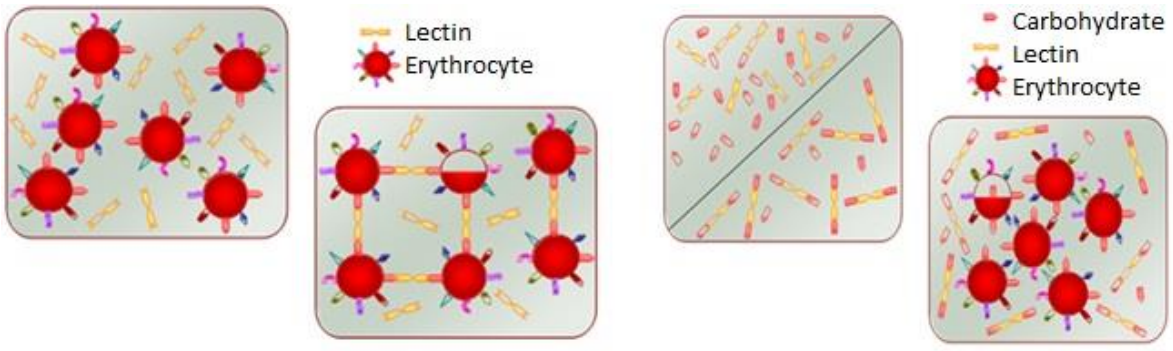


Figure 2: Hemagglutination assay: in Table 1 we see a solution of lectins and erythrocytes, in Table 2 we see the lectins binding to the surface carbohydrates of the erythrocytes promoting a network between them, the inhibition test of hemagglutinating activity (Table 3 and 4) In the presence of other free carbohydrates in solution confirms the hemagglutination potential of Lectins. Source: Adapted from Silva 2008.

6.6.2 Physical Chemical Assays:

There are several Physical Chemistry studies that can be done using purified lectins such as Mass Spectrometry, Structural Characterization, Ionic Force Variation Studies, Spectroscopic Characterization, Sequencing and Structure Analysis, Circular Dichroism, Fluorescence Emission Spectroscopy, Studies of Thermal stability, among others, the main assays are:

6.6.2.1 Thermostability:

Proteins denature in the presence of high temperatures, some lectins are thermosensitive, other thermostable, this means that such proteins have their activity optimized at certain temperatures and absent at unfavorable temperatures, is an important assay to evaluate the stability of the lectin at varying temperatures (Negreiros *et al .,* 2016 ; Trindade, 2006).

6.6.2.2 PH:

Another important evaluation is the stability at different pH values, because these proteins must be kept in solution that present ideal conditions that maintain their biological activity and consequent subsequent use in the experiments to which they can be submitted, since they can undergo denaturation at unfavorable pH, the pH has a varied effect on the lectins, in some cases does not affect the activity, in other cases causes denaturation as for example in the process of Elution by PH difference (Negreiros *et al .,* 2016 ; Trindade, 2006).

6.6.2.3 Electrophoresis:

Polyacrylamide gel electrophoresis (PAGE) can be performed using a sodium dodecylsulfate (SDS) gel, which under reducing conditions reveals the degree of purity, composition and molecular mass of subunits. The gels may be stained with coomassie blue or starch black detecting polypeptide bands, or with glycoprotein-specific staining, such as Schiff staining, another important electrophoretic method is the electrofocalization or isoelectric focusing, which determines the isoelectric point of the protein of interest. (Reynoso-Camacho *et al*., 2003).

6.6.2.4 Reversed Phase High performance Liquid Chromatography HPLC-RP

The HPLC-RP technique is a useful means of obtaining purer lectins that have already been purified, of estimating molecular weights, of characterizing and fractionating very similar proteins and peptides. HPLC-RP has been widely used as a final step of more refined lectin purification after the use of other chromatographic methods and as a method of characterizing the molecular mass of these proteins.

RP-HPLC have been used to establish the homogeneity of pure lectins, to separate subunit structures, as well as to determine whether these molecules are monomeric or not (Wang *et al*., 2000).

7 - BIOLOGICAL PROPERTIES

Lectins has the ability to bind monosaccharides and oligosaccharides, presents variety of useful biological effects, some of these effects serve as the basis for the application of lectins in the investigation of activities in the areas of Biochemistry, Cellular and Molecular Biology, Immunology, Pharmacology, Medicine, Biotechnology and Clinical Analyzes.

They are a valuable tool for the structural investigation of carbohydrate complexes, especially glycoproteins, for the analysis of changes that occur on the cell surface during physiological and pathological processes, can be used as reagents in mitogenic stimulation, can be used as affinity matrices for the purification of several glycoproteins and glycolipids (or any sample containing saccharides) of great medical importance, for example, the purification of glycoproteins from the virus of HIV, cell marker for diagnostic purpose, controlled drug release, isolation and purification of glycoconjugates by affinity chromatography (Bertrand et al., 1998 ; Macintyre et al., 2001; Takahashi et al., 2008; Sitohy et al., 2007; Kaur et al., 2006).

7.1 Lectins and applications in Immunology:

The MBL (mannose-binding lectin) is an acute phase protein found in human serum, secreted by the liver, is an important element of the innate immune system, which can activate the complement system (body defense system to opsonize pathogens and induce responses Inflammatory agents that fight infection) by the Lectin pathway, facilitating the phagocytosis of pathogens, MBL binds to mannose, glucose or other sugars from microorganisms such as bacteria (*Salmonella, Listeria* and *Neisseria*), pathogenic fungi such as *Candida albicans* and *Cryptococcus neoformans* and even some viruses such as HIV-1, several studies associate MBL levels with

disease, susceptibility and immunity. (Anna; Francisco 2003; Sitohy *et al.,* 2007; Santi-Gadelha *et al.,* 2006).

Some lectins are capable of acting on lymphocytes, causing such cells to pass from a quiescent state to a state of growth and proliferation. The lectin from *Aloe arborescens* (Koike *et al.,* 1995) and the lectin of *Cratylia mollis* (Maciel *et al.,* 2004) are some examples of lectins with mitogenic activity that can be used in *in vitro* assays.

7.2 Vegetable lectins and action against insects:

Insecticides are used to control pests that destroy crops, as well as species that transmit disease etiological agents, the use of some insecticides such as organophosphates and pyrethroids, causes environmental damage in addition to intoxication of vegetable consumers and rural workers. Some plant lectins has an insecticidal action, which makes it possible to use these proteins as a bioinsecticide, acting on insects that cause damage to the agricultural production, the lectin ligands of chitin are an example, therefore they are a promising ecological alternative, even though they are not as effective as synthetic chemical insecticides, their use minimizes the risks of side effects in humans beyond environmental damage. (Macedo *et al.,* 2007; Coelho *et al.,* 2007; Liu *et al.,* 2007).

7.3 Lectins and Cancer:

Also acting in inhibition of tumor cell growth, antitumor action has been observed on sarcoma 180 and on some human tumor cell lines, the modulating effect of the immune response is a property observed in some lectins, such as leukemia cells (L1210 and M1) and hepatoma (Hep G2), the mechanism of action of anticarcinogenic lectins consists of their function of binding to membrane carbohydrates in the membrane of the mutant cell or its receptors causing apoptosis by agglutination and tumor decrease. (Ngai , Ng, 2004; Petrossian *et al.,* 2007; Andrade *et al.,* 2004;

Karasaki *et al.,* 2001; Gavrovic-Jankulovic *et al.,* 2008; Kaur *et al.,* 2006; Kaur *et al.,* 2005).

7.4 Lectins and anti-HIV action:

The observation that lectin with antifungal activity isolated from *Phaseolus vulgaris* had a strong inhibitory action on HIV-1 protease is another example of the potential application of these proteins, especially those with specificity to mannose or N-acetyl-glucosamine, they also have an ability Anti-HIV and in cell culture assays not only inhibiting cell infection but also preventing viral infection of infected cells to uninfected T lymphocytes. Some lectins may also act by inhibiting HIV-1 reverse transcriptase activity, HIV-1 expressing gp120 and gp41 which are protein fusion receptors present on the viral surface, gp120 is highly glycosylated, has several carbohydrates in more than half its molecular mass, constituting a biological target for MBL - mannose-binding lectin (Zheng *et al.,* 2007; Wang, Ng, 2006 , Molchanova *et al.,* 2007; Balzarini, 2006).

7.5 Lectins and Clinical Analyzes:

In clinical analyzes, lectins are used to determine blood types and to diagnose processes of development, differentiation and neoplastic transformation, detection and separation of glycoconjugates (Paiva et al., 2006; Wroblewski et al., 2001; Zheng et al., 2007; Wang et.al 2007; Banerjee et al., 2004; Maciel et al., 2004).

The affinity of lectins for cell surface glycoproteins has been used for the identification of microorganisms such as, for example, *Neisseria gonorhoeae* that can be differentiated from other species of *Neisseria* (Wu , 2006) or for identification of ABO blood groups (Matsui et al., 2001).

Proteins isolated from plant tissues have shown strong antibacterial and antifungal activity. The ability of plant lectins to react with carbohydrates exposed on

the cell surface of microbes has made it possible to use these biomolecules as diagnostic probes for the identification of pathogenic bacteria, which are based on the reaction of selective agglutination between lectin and bacterium. Partially purified lectins from seven medicinal plants of South Africa were evaluated for the antibacterial effect against *Staphylococcus aureus* and *Bacillus subitilis* by means of agglutination method, showing inhibitory effect on the growth of the bacteria (Ordóñez et al. 2006).

Researchers analyzed the different patterns of bacterial agglutination promoted by 23 Lectins and showed that the interaction lectin vs. bacteria is a good tool to quickly identify *Mycobacterium* species. In addition, the antimicrobial activity of lectins stimulates the evaluation of them as new antibiotics (Wang & NG 2003; Wang & Bunkers, 2000, Wang et.al 2003, Ratanapo *et al*., 2001).

Lectins have the ability to bind specifically to fungal hyphae and act to prevent the consumption of nutrients and the incorporation of precursors necessary for fungus growth. They also act on the germination of fungal spores, probably at a very early stage of the process, inhibiting it, so that there is a prolongation of the latent period preceding germination, antifungal activity was observed in a lectin isolated from *Castanea mollissima* against fungi *B. cinerea, M. arachidicola* and *Physalospora piricola*, Lectins have also been used with great success as indicators of fungi, since these compounds are highly specific to the carbohydrates present in the cell wall of the same ones (Zabel & Morrell, 1992).

The knowledge of the saccharide profile on the fungal surface enables the use of lectins as cellular targets, which can serve as carriers of antifungal agents that use as specific targets the carbohydrates on the cell surface of the microorganism. (Wang & Ng, 2003).

Lectins are also useful in forensic medicine, characterizing cerebral pathologies (Uryu *et al.,* 2007), assisting in the investigation, prognosis or diagnosis of brain

disorders in postmortem circumstances in humans (Arendash et al., 2007; Uryu et al., 2007; Ishikawa et al., 2006) and in animals (Medina-Flores et al., 2004).

7.6 Lectins in Biotechnology and Pharmacology

According to De Mejía EG & Prisecaru VI, 2005, lectins digestion-resistant are able to maintain their conformation and functionality during their passage through the intestine are capable of binding the gastrointestinal cells or are able to reach intact the circulatory system, thus they are useful in the production of smart drugs, where these drugs differ of the traditional ones because they act in specific cells of the organism with more selectivity to specific receptors avoiding side effects as for example in the case of the medicines for cancer. Since tumor cells express glycans on their surface different from normal cells, lectins can be used as mediators in the transport of drugs through endocytosis in cells. (Thöm *et al.,* 2007; Woodley, 2001).

Since some lectins have the ability to mediate mucoadhesion, cytoadhesion and drug cytoinvasion (Gabor et al., 2004), these molecules have been exploited in drug delivery systems. Lectin of leaves of *Bauhinia monandra* and *Lens culinaris* were incorporated and also adsorbed on the surface of nanoparticles, showing to be potential tools in oral administration drugs, with controlled release (Rodrigues *et al.,* 2003).

8 – CHITIN BINDING LECTINS:

Chitin, $(C_8H_{13}O_5N)_n$ is a long chain polysaccharide of N-acetylglucosamine, whose properties have been exploited in industrial and biotech applications for almost seventy years, chitin binding lectins have been isolated from a variety of sources including bacteria, insects , plants and mammals and has antifungal and insecticidal activity. Chitin is the main component of the fungal cell wall and exoskeleton of insects, are resistant to proteolytic degradation, when ingested by insects, and inside the intestinal tract they interact with glycoconjugates present on the membrane surface of epithelial cells (Trindade *et al.,* 2006 , Sitohy *et al.,* 2007; Neumann *et al.,* 2004; Roberts 1992; Goosen 1996).

This family comprises all lectins which contain at least one domain equal to the hevein domain (*Hevea brasiliensis*) which is a chitin binding lectin, this category comprises Merolectins, Hololectins and different types of Chimeroctins. (Van Damme *et al.,* 1995).

The most studied chitin binding lectins are those of the Hevein family that have hevein domain responsible for the recognition of chitin, heveins possess 43 amino acids, are rich in glycine and cysteine residues, having 4 disulfide bonds are quite stable, being able to maintain their Biological activity even under 90 degree heating which makes it a thermostable protein. This family of lectins presents two potentially useful biological activities, the first is the insecticidal activity since the insects possess chitin in their digestive tract, several studies prove this property, for example, *Arisaema jacquemontii* lectin affects the development of *Bactrocera larvae* (Kaur et al., 2006).

The second important biological activity is the antifungal action, two proteins of *Malva parvifolia* have a potent antifungal action and presented a fungicidal action against the fungus *Fusarium graminearum* (Wang , Bunkers, 2000) and lectins isolated from Seeds of *Phaseolus* and *Adzuckia angularia* possess a potent antifungal activity

against the fungi *Botrytstis cineria, Mycospharella, arachidicola* and *Fusarium oxysporium* (Ye , Ng, 2001, Ye , Ng, 2005).

Problems caused by fungi (infections, food contaminants) and insects (disease transmission, natural predator of plantations) are a challenge for science, the search for new, more specific and selective molecules to combat them in a way that does not cause effects toxins in humans and the environment has been a challenge, so the development of research aimed at detecting, purifying and characterizing new chitin binding lectins has become a major ally in science in the search for compounds that can combat these problems.

8.1 Lectin chitin binder of *Mauritia flexuosa L. f.*

- Domain: *Eukaryota*
- Kingdom: *Plantae*
- Division: *Magnoliophyta*
- Phylum: *Tracheophyta*
- Class: *Liliopsida*
- Order: *Arecales*
- Family: *Arecaceae*
- Sub-family: *Mauritiinae*
- Genre: *Mauritia*
- Species: *Mauritia flexuosa* L.f.

(John T *et.al* 1995)

Figure 3 : Buriti (*Mauritia Flexuosa*) .

Source: adapted from Negreiros 2016

Most of plant lectins are purified from dicotyledons mainly from the *Leguminosae* family, monocotyledons lack further research studies that can characterize them chemically and biologically in order to discover new potential sources of lectins, the *Mauritia flexuosa* L.f popularly known as Buriti, is a monocotyledonous plant belonging to the family *Arecaceae* also known as palm tree, has tropical and subtropical distribution mainly in South America, reaches up to 25 meters in height, the fruit is a globose-elongate of 4 to 7 cm in length, constituted of epicarp formed of rhomboid scales of reddish brown color, the endocarp is spongy that surrounds the seed and the mesocarp is a thick mass of orange color. It has great socio-economic utility used as crafts, construction of houses, boats and bridges, gastronomy (oil, starch, wax, fibers, candys), wound healing and its oil has great use in cosmetology as moisturizers, oils and sunscreen (Endress, *et al.,* 2013; Lorenzi, *et al.,* 2010).

The biochemical laboratory of the Federal University of the São Francisco Valley – UNIVASF, carried out characterization studies of *Mauritia flexuosa's* crude extract , when Negreiros in 2016 evaluated the absense of hemagglutinating activity, that is, lectin specificity to binding sites of carbohydrates exposed on the surface of erythrocytes due mainly to hydrogen bonds and hydrophobic interactions. Since no haemagglutinating activity was observed in the tested samples and it is known that the lectins have carbohydrate specificity, in addition to agglutinating blood cells, they can agglutinate other cells depending on the carbohydrate exposed on their surface, the test was performed with agglutination using yeast cells *Saccharomyces cerevisiae* which is a yeast with a cell wall rich in chitin, where binding activity was observed, proving that the fruit probably has chitin binding lectins causing agglutination in the fungus. The control of the thermal stability of a protein represents an important strategy to maintain its biological activity, since unstable thermoplastic proteins are easily denatured by losing their biological activity. It was found by Van Damme, et al. (1995) that the monocotyledon lectins are quite stable to heat, the crude extract of “Buriti” was submitted to the heat treatment of autoclaving and it was proved the high thermal stability of these lectins that even after autoclaving for 20 minutes at 100C° still showed binder activity .

8.2 Lectin chitin binder of *Artocarpus incisa*

- Kingdom: *Plantae*
- Order: *Rosales*
- Family: *Moraceae*
- Genus: *Artocarpus*
- Species: *Artocarpus incisa*

(John T et.al 1995)

Figure 4: Artocarpus incisa.

Source: United States Department of Agriculture. 2007

The genus *Artocarpus (Moraceae)* comprises about 50 species of trees, is of great scientific interest because they contain important secondary metabolites and useful biological activities, originating in the tropical forests of India was distributed to the East African, Brazil, Philippines and Suriname, adapted to the tropical weather is cultivated in humid environment without much special care, it consists mainly of two species called *Artocarpus integrifólia* (popular name: Jaca) and *Artocarpus altillis* also known *as Artocarpus incisa* (popular name: Bread Fruit) that inhabit tropical rainforests, the name *Artocarpus* derives from the Greek words 'artos' (bread) and 'karpos' (fruit) (Monteiro Moreira 2002).

Lectins of the genus *Artocarpus* have begun to arouse scientific interest since Monteiro-Moreira discovered in *Artocarpus integrifolia* a galactose ligand lectin called Jacalin with properties of binding to human IgA and anti-HIV action, this stimulated the research and isolation of new lectins from two main species of this genus. Later, another lectin of *A.integrifolia* called ArtinM, which had D-mannose affinity and was able to stimulate neutrophil migration and has a high healing power for burns, is

currently being studied for the production of topical medicinal products based on this lectin to stimulate cicatrization, and finally a chitin binding lectin called Jackin was isolated. (Monteiro-Moreira 2002).

Previous studies in *A.integrifolia* stimulated research on bread fruit lectins (*Artocarpus incisa*) where it was found to have a amino acid sequence similar to lectins of *A.integrifolia* ,the most abundant lectin in *A.incisa* is" Frutalin" which is a multilectin with affinity for D-galactose and has 98% of similarity with "Jacalin" from *A.integrifolia*, the second most abundant lectin is "Frutapin" which is homologous to "ArtinM" , both have D-mannosis binding site and has been successfully cloned and expressed in *Escherichia Coli.* (Monteiro-Moreira 2002,)

Monteiro Moreira detected in the saline extract of *A.integrifolia* and *A.incisa* two new chitin binding lectins, they were isolated and their N-terminal polypeptide chains were determined, showing that this new lectin had no similarity with the previously sequenced lectins, and were denominated "Jackin" and "Frutackin" respectively. Trindade characterized chemically these two unpublished lectins and their studies revealed that they are homologous to each other, having monomers of 14kDa formed by three subunits, joined by S-s bridges, have 62% similarity, are rich in cysteines, amino acids. Since Frutackin has already been chemically characterized, it needs further studies of its biological activity since it is a lectin binding agent of chitin and may be a therapeutic alternative to conventional antifungals and insecticides. *Artocarpus incis*a has two varieties, Bread Fruit of dough (no seeds) and Bread Fruit with seeds, has yellowish pulp, pasty texture, consumed in European, Canadian and Mexican (Monteiro-Moreira 2002).

9 - FUNGI

The Fungi kingdom has eukaryotic organisms with membranes containing steroids, and ribosomes type 80S, is composed of yeasts, molds and mushrooms, the cells of fungi or hyphae, differs from the other eukaryotes because it contains cell wall composed of chitin and glicans, in reverse of plant cells, which contain cell wall composed of cellulose and human cells that do not contain cell wall (Mueller & Schmit , 2006 ; Hawksworth , 2006 ; Harris 2008).

They are eukaryotic, protist, non-photosynthetic beings, their cellular structure has similarity to human cells, they have nuclear membrane that surrounds the chromosomes and the nucleolus, it has organelles such as the endoplasmic reticulum, golgi complex, mitochondria and vacuole, the cell wall Is composed of hexoses and hexoamines, which form mannans, ducans and galactans, most of which have a wall rich in chitin (N-acetylglucosamine), others have polysaccharide and protein complexes, predominantly cysteine. They are divided into macroscopic and microscopic fungi and presents as filamentous and/or yeast fungis. (Cooke M.C, Chapter I,II,II , 2009; Ximenes et. al 2009).

They are found almost everywhere on earth, some (saprophytes) live on organic matter, water and ground, and others (parasitic fungi) live on the surfasse of ground or inside animals and plants. Some are harmful while others are beneficial, fungi also live on many usable materials causing spoilage and spoiling food. (Mueller & Schmit , 2006 ; Hawksworth, 2006).

The microscopic morphology of fungi shows that most of the fungi develop as hyphae which are filamentous and cylindrical structures,with 2µm to 10 µm in diameter and up to several centimeters in length, the hyphae are specifically adapted to growth on solid surfaces and to the invasion of substrates and tissues, these hyphae grow and form new hyphae by branching or bifurcation of their ends giving rise to two new hyphae, successive hyphae forming a mycelium , which is an interconnected network

of hyphae. Mycelium of fungi may become visible to the naked eye on various surfaces and substrates such as wet walls and spoiled food, commonly called molds. (Wang ZY *et.al.,* 2005).

Because fungi can not use solar energy to synthesize their own food, they are considered heterotrophic organisms that take out nutrients (such as nitrate, ammonia, acetate, or ethanol) that need a substrate, which may be dead organisms or living plants, The pathogen *Magnaporthe grisea*, for example, forms a structure called appressorium that has evolved to pierce plant tissues and withdraw its nutrients(Howard *et al.,* 1991).

Reproduction of the fungi can be asexual (by means of vegetative spores called conidia, or through fragmentation of the mycelium) or sexed (meiosis), and these reproductive characteristics can be classified into seven phyla: *Microsporidia, Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, e Basidiomycota.* Vegetative reproduction occurs by budding or fission (transverse division, followed by separation of the daughter cells) or fragmentation of the hyphae, in the reproduction with spores (asexual) the fungi produce spores of the aplanospores type, inside sporangia (endospores) or in the end of Sporangiophores (exospores), gametic (sexed) reproduction is the least common but is important for genetic variability. (Hibbett DS; et al. 2007).

They are used in the food industry (mushrooms, fermentation of breads, wine, yoghurts, beer and cheeses), in the pharmaceutical industry (production of antibiotics), biotechnology (fermentation and enzyme production), organic matter decomposition like decomposers of lignins or cellulose and recycling of nutrients in forests. (Chang S-T & Miles PG, 2004 ; Hawksworth , 2006).

For their growth, they need to use micronutrients from other organisms like iron, zinc, copper, manganese, boron, cobalt and molybdenum, as well as vitamins such as thiamine (B1), pyridoxine (B6) and riboflavin (B2), although a few Species synthesize their own vitamin, as for temperature, most fungi grow well between 0ºC and 35ºC, but

the optimum is in the range of 20ºC to 30ºC. As for pH, fungi prefer acidic for their growth, leaving the optimum in the Around 6, light is not important for its development, but a little light is essential for the occurrence of sporulation in many species. Light also plays a part in the dispersion of spores, and the sporangia of many fungi are positively phototropic and discharge their spores into light (Chang S-T & Miles PG, 2004 ; Hawksworth , 2006).

They produce secondary metabolites called mycotoxins that allow them physiological adaptations, competition with other microbes and fungi and protection, normally healthy and immunocompetent individuals present high innate resistance to fungal infection, although they are constantly exposed to the infectious forms of various fungi present as part of the microbiota (Endogenous) or in the environment (exogenous) (Keller NP *et.al* 2005; Demain AL & Fang A. 2000).

Many fungi live in a harmonious way in our body, however, situations that cause their overpopulation (immunodepressed people) can cause diseases such as candidiasis and pityriasis versicolor , both are mycoses, which is the result of too much and uncontrolled proliferation of these organisms in the epithelium, in some cases, the same agents of cutaneous infections, may migrate and colonize differentiated regions, such as the respiratory, nervous, genital and gastrointestinal systems, the treatment of fungal diseases is usually more time-consuming than a bacterial infection and the chances of recurrence rates are also higher (Konemam & E.W.; Roberts 1992; Warren Levinson & Ernest Jawetz, 2005).

Like human cells, fungi are eukaryotes with chromosomal DNA inside the cell nucleus and also have endoplasmic reticulum, golgi complex and mitochondria, share similar mechanisms of DNA replication and protein synthesis, this similarity with human cells is a barrier to The development of new drugs that are selectively toxic to fungal cells and do not affect human cells. Knowledge about the cellular structure of the fungus and its function is essential to understand the pharmacology of antifungal and to

develop new drugs with a more selective therapeutic aim with greater effectiveness and fewer side effects, antifungal drugs currently act in four possible ways In fungi: in their membrane, in their cell wall, in DNA / RNA synthesis or in inhibition of mitosis. (Zabriskie & Jackson , 2000, Desjardin *et.al* 2008).

Risk factors for developing infections by these three opportunistic fungi are: therapies that suppress the immune system, immunosuppressive drugs such as corticosteroids or chemotherapy, Leukemia, Hodgkin's disease and lung disease such as emphysema (Keller et.al 2005).

The environment has many spores of various fungi, and these usually float in the air. Among the wide variety of spores that land on the skin or are inhaled into the lungs, some can cause small infections, which rarely spread to other parts of the body. A few types of fungus (like *Candida*) can live normally on the surface of the body or in the intestines. Only occasionally do these normal inhabitants of the body cause local infections of the skin, vagina, or mouth, however, in rare cases, they cause greater damage. Sometimes certain fungal species can cause severe infections of the lungs, liver and the rest of the body, the intact skin is an effective defense of the host against certain fungi (for example *Candida* and dermatophytes), but if the skin is damaged, organisms can settle. Fatty acids in the skin inhibit the growth of dermatophytes and hormone-associated skin changes in puberty limit scalp mycosis caused by *Trichophyton*. The normal flora of the skin and mucous membranes prevents the appearance of fungi. When normal flora is inhibited, for example by antibiotics, excessive growth of fungi like *C. albicans* can occur (Campisi *et al.,* 2002).

There are opportunistic fungal pathogens such as *Candida albicans* (cause candidiasis) *Cryptococcus neoformans* (a pathogen that causes cryptococcosis which is a systemic infection that can reach the lungs or meninges) and *Aspergillus fumigatus* (responsible for Aspergillosis that causes lung infection evolving to systemic infection), which only cause infections when there are breaks in the protective barriers of the skin

and mucous membranes or when there are defects in the immune system of the host, usually infect Immunocompromised patients such as patients with AIDS or in treatment with chemotherapeutic agents, this type of patient presents infections caused by fungi that hardly cause harm to individuals who have a normal immune system. (Campisi et.al 2002 ; Magliani et.al 2005)

9.1 *Candida albicans*

Naturally present in the human body, in normal people it does not cause infections, but in special cases such as pregnancy, diabetes and impaired immune system as in AIDS, can proliferate excessively and invade tissues causing candidiasis, which are local infections of the skin, vagina or of the mouth and can spread to the whole body mainly in cases of leukemia with low leukocyte count and surgeries and other invasive procedures and may also cause endocarditis. The symptoms of candidiasis vary depending on the infected tissue, on the retina it can cause blindness, in the mouth produces creamy, white and painful plaques, the diagnosis is based on the culture of samples of blood or cerebrospinal fluid and through the symptoms. (Mazneikova 2003 , Campisi G et.al 2002).

Candida albicans has three metabolic pathways: the classical respiratory chain (cytochrome pathway, CRC), parallel respiratory chain (PAR) and alternative ubiquinol oxidase (AOX), these metabolic pathways facilitate the growth of these pathogens, since when antifungal therapies are used conventional drugs, these drugs can act in a certain way and yet the yeast continues to develop, as it has the other two alternative routes to maintain its energy metabolismo. With the knowledge of these pathways and the antifungal agents that inhibit them, it is possible to elucidate the mechanism of action of novel antifungal agents with more selective therapeutic target. (Ruy *et al.* 2006)

The most commonly used drugs in the treatment of *Candida albicans* infections are: nystatin, miconazole, intraconazole, amphotericin B, and voriconazole, usually oral treatment with fluconazole or Itraconazole, may be associated with topical creams such as nystatin, ketoconazole and nitrate of miconazole, in cases of systemic infection it is treated with intravenous amphotericin B (Santos *et al.,* 2005 , Olnick 1980, Gregory 1996, Gualco *et al.,* 2007)

Candida albicans is the main cause of these infections, but they are also known to cause infections, such as *Candida tropicalis, Candida glabrata, Candida krusei, Candida parapsilosis, Candida kefyr, Candida guilliermondii* and *Candida lusitanae*, the most common form Is the oral candidiasis mainly in patients with HIV virus. This is because the virus destroys the TCD4 + lymphocyte, which participates in the immune system in defense of the organism against invading agents, there are other forms of infection such as the onychomycosis that is the proliferation of this yeast insidde nails and vulvovaginal candidiasis, characterized by inflammation in the vagina with dense and milky discharge, intense irritation and pruritus, caused by the decrease in the vaginal pH that predisposes the disease. (Campisi et al., 2002, Gregory 1996, Sobel 2004).

The most used diagnostic culture medium in the primary isolation of *Candida* is Sabouraud dextrose agar (ADS), as it allows the growth of yeast and inhibits the growth of other bacteria due to acid pH. Typically, ADS cultures are incubated in aerobiosis at 37 C° for 24-48 hours. Under these conditions, the colonies are usually smooth or wrinkled, creamy and white-yellowish (Gualco *et al.,* 2007, Campisi 2002)

The pathogenicity of *Candida albicans* is due to the formation of hyphae that exert mechanical strength helping in the adhesion and penetration into the host cell, allowing it to invade tissues, many anti fungal have undesirable side effects besides some strains are resistant to them, the search for new molecules more selective and

with fewer side effects constitutes an important therapeutic alternative against this pathogen (Santos *et al.,* 2005, Gualco *et al.,* 2007).

9.2 *Cryptococcus neoformans*

Etiologic agent of cryptococcosis, an infection of systemic involvement, central nervous system and more commonly pulmonary, is an oval yeast, with characteristic capsule of mucopolysaccharide, has white to cream colony, bright, mucoid texture, smooth and whole margin after three days at a temperature of 25 to 37 ° C, in culture medium such as Agar Sabouraud 2% glucose and agar extract of malt and yeast, the predominant capsular component is glucuronoxylomannan, among the main virulence factors of *Cryptococcus neoformans* are thermotolerance, cell wall and capsule components, adhesion capacity, hormone receptors and the production of enzymes. (Kurokawa *et al.,* 1998 , Kwon-Chung KJ *et al.,* 1984, Magliani *et al.,* 2005, Shoham *et al.,* 2005).

The production of melanin that is deposited on the wall of the fungus comes from substrates containing dopamine and from the action of catalytic enzymes such as phenoloxidase. The brain is rich in substrates for phenoloxidase such as dopamine and could be responsible for the propensity of these organisms to infect the nervous system causing cryptococcal meningitis.. (Kurokawa *et al.,* 1998 , Kwon-Chung *et al.,* 1984, Magliani *et al.,* 2005, Shoham *et al.,* 2005).

C. neoformans inhabits bird feces as the pigeon, the bird does not develop the disease by having a body temperature around 42 ºC, by inhaling the fungus present in soils containing bird droppings, it penetrates the lungs and can develop and disseminated throughout the body, especially in immunocompromised patients, since it is an opportunistic disease, transmission does not occur from person to person. (MacDougall et.al 2007)

The treatment is made with polyphenolic antifungals (amphotericin B), 5-fluocytosine and azoles (fluconazole, itraconazole, voriconazole and posaconazole),

the United States Infectious Diseases Society recommends that this first line of attack be made with the combination of two antifungals , Amphotericin B (1 mg / kg / dose, IV, does not exceed 50 mg / day for 6 weeks) and 5-flucytosine (5FC), but the latter is not available in many countries, Fluconazole (200-400 Mg / day, VO or EV for approximately 6 weeks, or associated with amphotericin B until cultures are negative) is used in AIDS patients to suppress long-term cryptococcal meningitis (Baddour et al., 2005 , Bennett JE et.al 1979 , Benson CA et.al 2004, Bicanic et.al 2006 , Bicanic et.al 2008)

For its cultivation it can be isolated from biological materials such as: cerebrospinal fluid, sputum, nodal pus, exudates of cutaneous and mucosal lesions, urine and blood, grow well in: Blood agar, Agar-Sabouraud and Agar heart- Between 25C° and 37C°, but grows best at 30C°, the maximum thermotolerance is 40C°. Membrane colonies of cream shade can be observed in 48h of incubation. The ability to utilize glycine as a source of carbon and nitrogen and resistance to blue canavaline of bromothymol allows the separation of *Cryptococcus gattii* from *Cryptococcus neoformans* in the medium. Blood culture may reveal the presence of fungus in the blood in disseminated disease (Bicanic T et.al 2008, Baddour LM et.al 2005, MacDougall L et.al 2007).

9.3 *Aspergillus fumigatus:*

They are filamentous fungi that grow in deteriorating vegetation, producing chains of conidia, the transmission occurs by conidia charged by the air where they settle in the host, cause infections in the skin, eyes, ears and especially in the lungs. In bronchopulmonary aspergillosis the mycelia grow in the form of balls, called aspergillomas, usually asymptomatic in normal people, except for cough with expectoration, cough and shortness of breath, in patients with AIDS the immune

system is compromised and the fungus is not controlled in the lung and Spread to other organs such as the brain and heart (Ascioglu S et.al 2004, Chamilos G et.al 2006).

A study conducted by Microbiology Researcher Ricardo Araújo at the Faculty of Medicine of the University of Porto (FMUP) showed that *Aspergillus fumigatus* is the pathogenic fungus most frequently found in the hospital environment, responsible for most opportunistic infections, aspergillosis (usually resulting from infection by *Aspergillus fumigatus*) is currently one of the infections with the highest mortality rates, reaching 85% even after administration of antifungal therapy, although some of the drugs administered to critically ill patients increase the germination and growth of the fungus and reduce the effectiveness of the antifungal drugs such as triazoles (itraconazole or voriconazole) usually treatment consists of the combination of corticosteroids and itraconazole, are clinical signs for diagnosis: peripheral eosinophilia, elevation of IgE, blood culture, percutaneous transthoracic biopsy and biopsy videothoracoscopy, galactomannan detection, cell wall polysaccharide (Araujo et al., 2004, Walsh et.al 2008, Barnes PD et.al 2006, Samarakoon P et.al 2008).

10 - JUSTIFICATIONS AND RELEVANCE OF THE STUDY

Just as fungi can be very useful to human life, being employed for various purposes like in the drinks, food and pharmaceutical industry, they can cause great damage to human health mainly through opportunistic infections in immunocompromised patients. Because of the similarity of fungal cells to human cells, some conventional antifungals have side effects due to lack of selectivity to the therapeutic target, and some fungi develop resistance to these drugs, the search for new molecules with a more selective therapeutic target becomes necessary for creation of new molecules with more pharmacological potency and fewer side effects.

Since the lectins of *Artocarpus incisa*, and *Mauritia flexuosa Lf* demonstrated interactions with the chitin column in affinity chromatography, it is important to investigate the biological activity of these new lectins against opportunistic pathogenic fungi in immunocompromised patients, since chitin is present in cell wall of fungi and is not present in human cells.

The investigation of the antifungal activity of these lectins may result in the discovery of new biomolecules that will be useful in antifungal therapy, especially at risk groups such as patients immunocompromised with AIDS or chemotherapy where they already use other drugs that also cause several adverse effects. The objective of this study is to investigate the thermodynamic properties, agglutinants and antifungal potential of these two new chitin binding lectins as a possible source of new, more selective drugs.

11 - GOALS

General : To purify chitin binding lectins from *Artocarpus incisa* and *Mauritia flexuosa L.f* and to test their antifungal activity in *Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus*.

Specific:

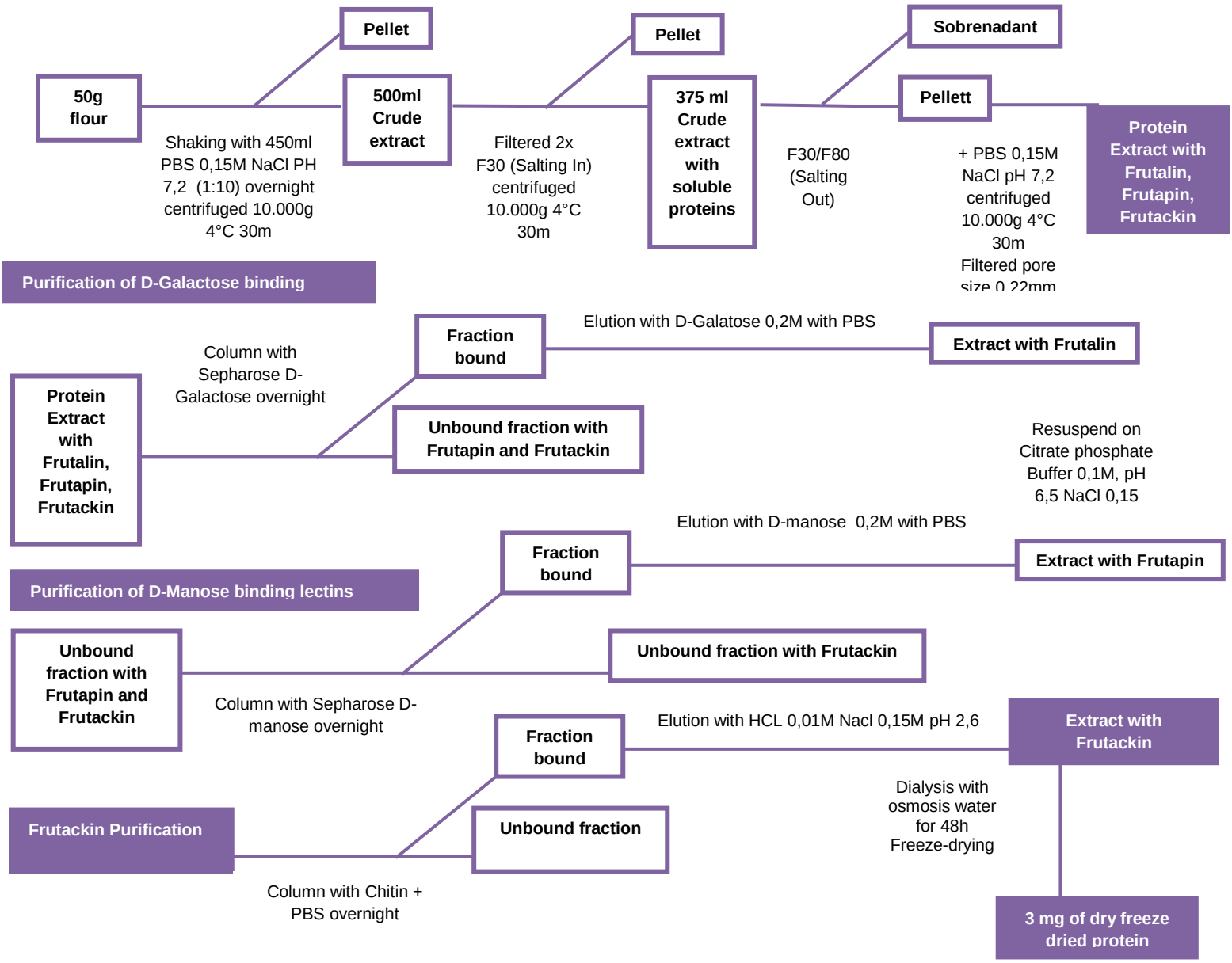
- Extraction, Isolation and Purification of lectin chitin ligand from *Artocarpus incisa* and *Mauritia flexuosa L.f* by affinity chromatography
- Establish optimum conditions for purification of this lectin, such as ideal pH, dialysis time, time in affinity chromatography, better eluent, better buffer
- To evaluate the agglutinating activity of these lectins
- To evaluate the thermostability of these lectins
- Evaluate its purity in electrophoresis
- Compare the performance of the two lectins
- To evaluate the inhibition of the growth inhibitory activity of opportunistic fungi of scientific interest such as *Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus*

12 - EXPERIMENTAL STRATEGY:

12.1 Assays in *Artocarpus incisa* extract*:*

The lectin chitin binding Frutackin was isolated from the crude aqueous extract of the *Artocarpus incisa* seeds using appropriate buffer, through successive affinity chromatographies in other columns with other carbohydrates to deplete other more abundant lectins such as Frutalin and Frutapin and then isolated in a matrix with chitin column and eluted by pH difference. After further purification the protein extract containing only the chitin binding lectin (frutackin) was subjected to assays

- Electrophoresis to evaluate protein purity and size
- Agglutination test in *S.cereviseae*
- Anti fungal assays in *C.albicans, C. neoformans and A. fumigatus*



Electrophoresis SDS PAGE

Aglutination in S. cereviseae

3 plates with *S. cerevisiae* + 0.15M NaCl (negative control)

3 plates with *S. cerevisiae* + 6mL Extract with Frutackin (positive control)

Anti fungal assay

C.neoformans

3 plates:

Positive control (50 µL of Fluconazole 5mg/ml)
Associated positive control (25 µL fluconazole 5mg/ml + 25 µL lectins 5mg/ml)
negative control (Nacl 0,15M)
lectin binding of chitin 1 (50 µL lectins 2,5mg/m
lectin binding of chitin 2 (50 µL lectins 5mg/ml)

C.albicans

3 plates:

Positive control (50 µL of Fluconazole 5mg/ml)
Associated positive control (25 µL fluconazole 5mg/ml + 25 µL lectins 5mg/ml)
negative control (Nacl 0,15M)
lectin binding of chitin 1 (50 µL lectins 2,5mg/mL)
lectin binding of chitin 2 (50 µL lectins 5mg/ml)

A. fumigatus

3 plates:

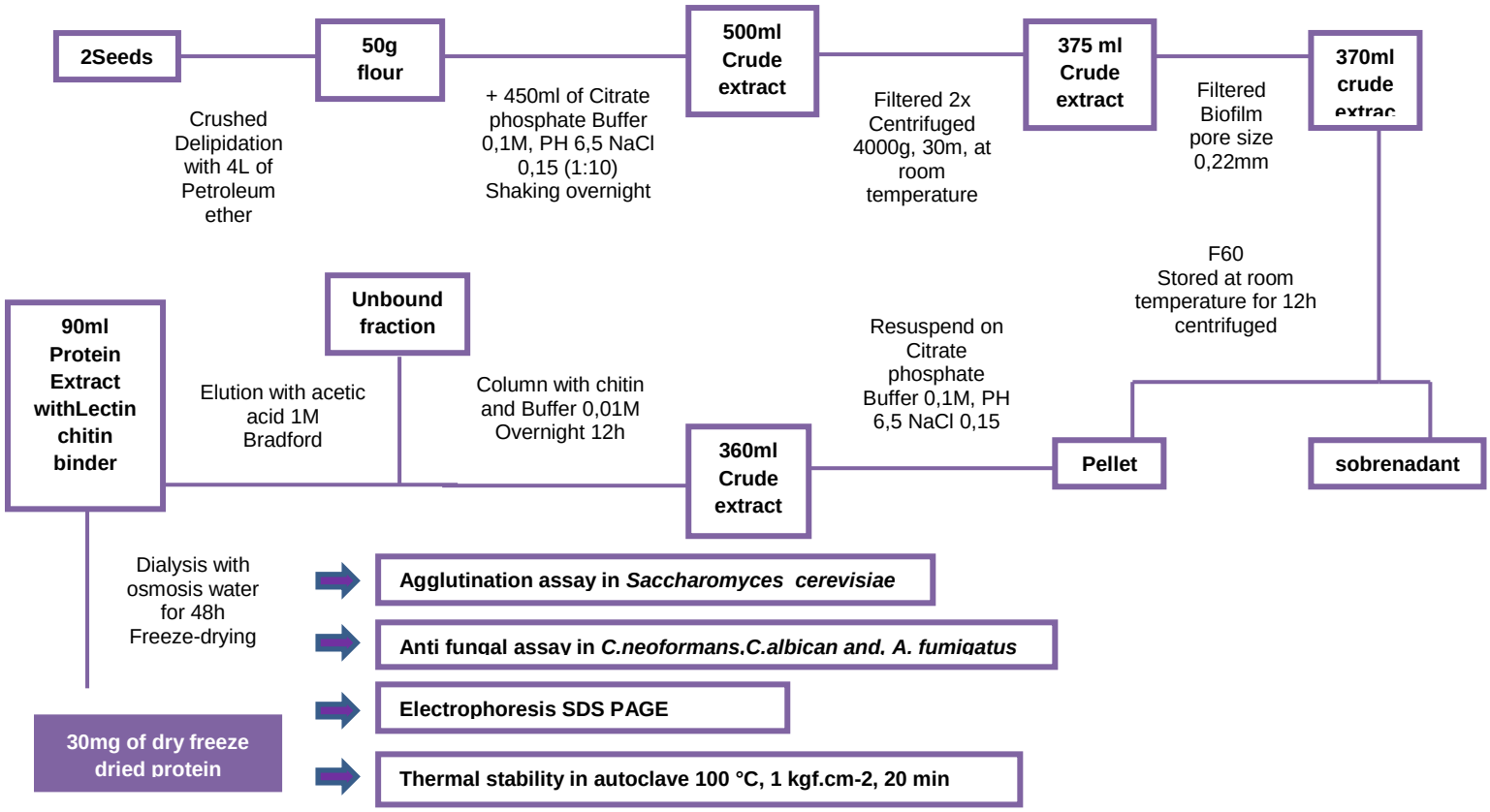
Positive control (50 µL of Fluconazole 5mg/ml)
Associated positive control (25 µL fluconazole 5mg/ml + 25 µL lectins 5mg/ml)
negative control (Nacl 0,15M)
lectin binding of chitin 1 (50 µL lectins 2,5mg/mL)
lectin binding of chitin 2 (50 µL lectins 5mg/ml)

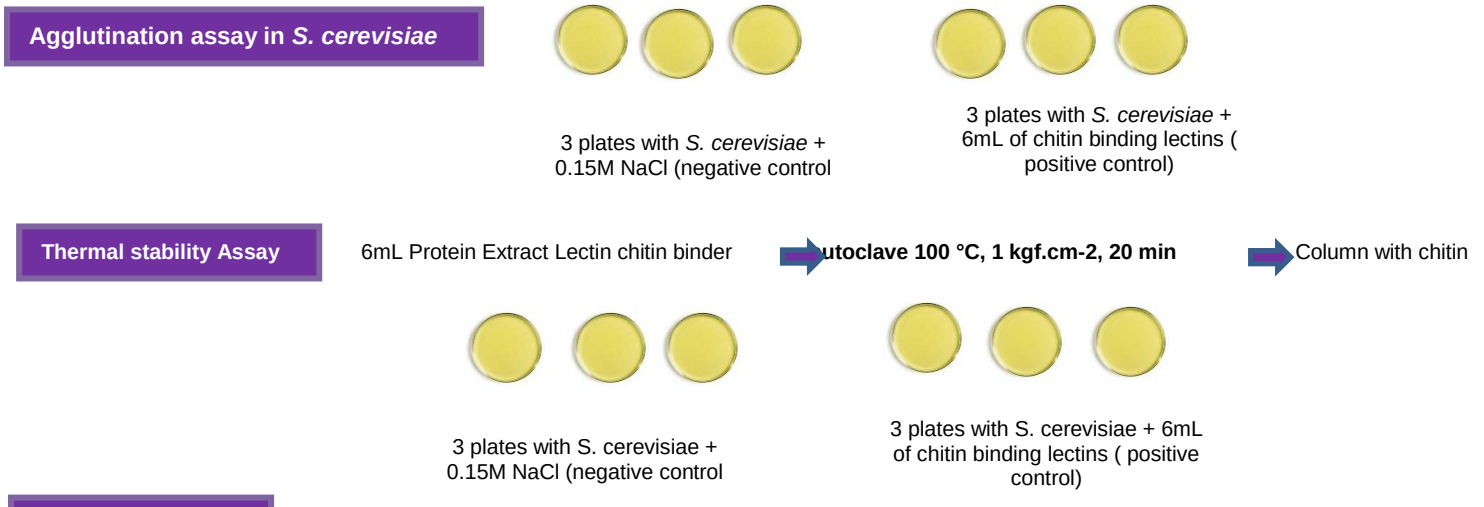
Figure 5 : Frutackin purification and assays Chart

12.2 Purification of Mauritia flexuosa L.f:

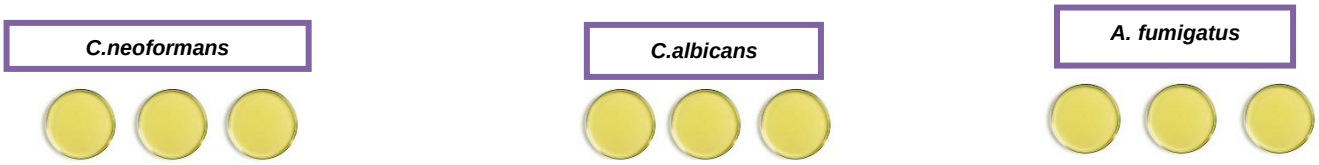
Buriti seeds were ground and passed through the delipidation process to remove excess lipids in the sample and facilitate the purification process, then purified by applying a crude extract directly to a chitin column matrix and eluted by pH difference. The assays performed with the *M. flexuosa* extract were:

- Purification of chitin binding lectins from crude extract
- Electrophoresis to evaluate protein purity and size
- Agglutination test in *S.cereviseae*
- Termostability test
- Anti fungal assays in *C.albicans, C. neoformans and A. Fumigatus*





Anti fungal assay



C.neoformans

3 plates:

Positive control (50 µL of Fluconazole 5mg/ml)
Associated positive control (25 µL fluconazole 5mg/ml + 25 µL lectins 5mg/ml)
negative control (Nacl 0,15M)
lectin binding of chitin 1 (50 µL lectins 2,5mg/m
lectin binding of chitin 2 (50 µL lectins 5mg/ml)

C.albicans

3 plates:

Positive control (50 µL of Fluconazole 5mg/ml)
Associated positive control (25 µL fluconazole 5mg/ml + 25 µL lectins 5mg/ml)
negative control (Nacl 0,15M)
lectin binding of chitin 1 (50 µL lectins 2,5mg/mL)
lectin binding of chitin 2 (50 µL lectins 5mg/ml)

A. fumigatus

3 plates:

Positive control (50 µL of Fluconazole 5mg/ml)
Associated positive control (25 µL fluconazole 5mg/ml + 25 µL lectins 5mg/ml)
negative control (Nacl 0,15M)
lectin binding of chitin 1 (50 µL lectins 2,5mg/mL)
lectin binding of chitin 2 (50 µL lectins 5mg/ml)

Figure 6 : *Mauritia flexuosa* assays chart

13 - MATERIALS AND METHODS

13.1 Protocol of purification of Frutackin of the seeds of *Artocarpus incisa*

The purification of Frutackin from the seeds of *Artocarpus incisa* was carried out in the Laboratory of Pharmaceutical Development of the University of Fortaleza - Unifor under the guidance of Professor Ana Cristina de Oliveira Monteiro Moreira who is a professor of the PhD in Biotechnology of the Northeast Network of Biotechnology - RENORBIO , Using the reagents, matrices and equipment of the institution itself. Chitin-ligand lectin was purified according to Monteiro-Moreira et al [Monteiro-Moreira 2002] methodology using the same procedures and solutions.

13.1.1 Solutions used in the purification of Frutackin

- phosphate buffer saline (PBS): 0.06 M dibasic sodium phosphate and 15 M sodium chloride pH 7.4 adjusted with 0.15 M monobasic potassium phosphate;
- glycine / 0.1 M chloric acid buffer pH 2.6 + 0.15 M sodium chloride;
- 0.2 M D-galactose in PBS;
- 0.2 M D-mannose in PBS;
- 0.15 M sodium chloride / hydrochloric acid buffer pH 2.6.

13.1.2 Materials used for purification of frutackin:

- Spectrophotometer mod U-2201 (Hitachi);
- Common refrigerated centrifuge (mod 5B and RC5C plus, Sorval) and bench (Modem 5414D Eppendorf and mod 206-R excelsa baby-II, Fanem);
- Savv Savant centrifugal / lyophilizer system (Speedvac);
- System for electrophoresis (Bio-rad);

- Conventional analytical balances, vacuum pumps, refrigerators and freezers (-20º C).
- Cross-linked galactomannan matrix extracted from *Adenantera pavonina* endosperm
- Sepharose D-galactose and Sepharose D-mannose (Pierce) matrix;
- Matrix of Chitin (Sigma-Aldrich);
- Other items: Amicon system for dialofiltration; Membranes, centricon, centriprep and YM dialysis bags (cutoff 3.5 kDa, Amicon Corp.).

13.1.3 Origin of plant material

- **Plant material:** *Artocarpus incisa L.* seeds were obtained from mature fruits from the city of Maranguape in the state of Ceará, Brazil, collected between September and November 2016.

13.1.4 Obtaining the crude extract of breadfruit seed flour

The seeds of A. incisa were removed from the fruit, washed and dried in an oven for 48 h at 37 ° C, then crushed in a common blender, sieved and the flour obtained was wrapped in identified flasks.

The flour was resuspended and diluted 1:10 in 0.15 M PBS buffer with NaCl pH 7.2 under constant stirring at room temperature for 4 hours. The suspension obtained was centrifuged at 10,000 g for 30 minutes at 4C° to prevent denaturation and degradation by proteolytic enzymes, the supernatant was then filtered on standard filter paper. Further purification was carried out using different fractions with Ammonium Sulfate.

Saltin In: In the “Saltin In Effect” where the solubility of the proteins is increased by the addition of small amounts of Ammonium Sulphate, the F30 fractionation with Ammonium Sulphate was applied to the filtrate, completely solubilized for 30 minutes and stored in a refrigerator for 1 Hour, then centrifuged again at 10,000g, 4°C for 30 minutes, the protein supernatant was recovered and the precipitate discarded.

Efeito Salting Out: In the supernatant F30 / F80 fractionation was applied until complete solubilization and stored in the refrigerator again for 1 hour and then centrifuged at 10,000g, 4C°, for 30 minutes, this time the supernatant was discarded and the precipitate obtained was resuspended at the lowest possible volume after solubilization ,after solubilization completely returned to the centrifuge under the same conditions (4°C, 10,000 g for 30 minutes) and the supernatant was filtered in a 0.22 µm membrane filter, obtaining the protein extract that will be used in the chromatographies.

13.1.5 Affinity Chromatography:

A protein affinity-based technique specifically binds to a particular linker, in which case the lectin which is the protein will bind specifically to the linker which is the carbohydrate, that specificity is useful in the construction of columns where the linker is covalently linked in a matrix wherein only those proteins that bind to the ligand of the column will be retained. In the purification of the frutackin, it will be necessary to deplete other lectins present in the seeds of A. Incisa in order to obtain a purer sample, the depletion begins with the more abundant lectins such as Frutalin and Frutapin, finally after the depletion of these, starts the purification of the frutackin.

Purification of D-galactose ligand lectins: The crude extract was applied in 12 mL aliquots on the galactomannan column (25 mL resin), this system was washed with PBS and the unbound material was collected and pooled. The columns were separated and the material bounded on the galactomannan column was eluted with

0.1 M glycine / HCl buffer pH 2.6. These materials that were attached and that were collected are Frutalin, from *A incisa*. Proteins were stored at -20 ° C, and the protein extract containing Frutapin and Frutackin submitted the next affinity chromatography.

Purification of the D-mannose ligand lectins: The unbound fraction from the previous step was applied to a sepharose-D-mannose column (10 mL resin). The column was washed with PBS and the unbound fraction collected. The bound material, Frutapin, was eluted with 0.2 M D-mannose in PBS and stored at -20 ° C, and the protein extract with chitin binding lectin called frutackin, submitted to the next chromatography**.**

Purification of the chitin-binding lectins: The unbound fraction from the previous step was applied on a chitin column (12 ml) equilibrated with PBS. The unbound fraction was discarded and the bound fraction was eluted with 0.01 M HCl / 0.15 M NaCl pH 2.6, which was immediately adjusted to pH 7.4, monitored in pH meter, with NaOH solution. This fraction was washed with PBS and concentrated 30 times by dialyfiltration in Amicon system using YM 3.5 membrane and monitored at 280 nM with collection of 1 mL fractions**.**

13.1.6 Electronic Absorption

Electron absorption spectroscopy for *in vitro* protein characterization is mainly used to determine concentrations, since there is a direct relationship between sample concentration and absorbance (A) or optical density, according to Lambert-Beer law, the reading is Made of 0.5mL quartz cuvette because this material does not interfere with the colorimetric method, the readings were made at = 280 nm, which are the absorption wavelengths of proteins with amino acids like tyrosine and tryptophan.

13.1.7 Polyacrylamide gel electrophoresis in the presence of SDS and tricine drag buffer (tricine SDS-PAGE) and protein dosage.

Electrophoresis (SDS-PAGE) is a protein separation technique that occurs through the differentiated migration of charged proteins by the action of an electric field by a polyacrylamide gel, is used to evaluate the degree of purity of a protein, in this experiment Gels of 16% SDS-PAGE and tricine buffer (0.1 M tricine in 0.1 M Tris and 0.1% SDS) were used to obtain better resolution of small proteins (5 to 20 kDa) according to methodology described by Monteiro Moreira et al. 2002. Protein samples were dissolved in buffer containing dye (bromophenol blue) in the presence and absence of β-mercaptoethanol, boiled for 5 minutes and applied to the gel. The runs were performed at 30 V for 1 hour (or until the proteins exited the stacking gel) and at 100 V for another 4 hours. The gels were stained with 0.05% Coomassie Brillant Blue 250 R and decolorized with a solution of acetic acid / methanol / water (1: 3: 8). The high molecular weight (MM) markers were BSA, OVA, ACB, ITS, CIT-C and the low molecular weight markers were fragments of myoglobin (153 amino acids): fragments 1 to 131; 56 to 153; 56 to 131; 1 to 55 and 132 to 153.

13.1.8 Agglutination Assay:

The purification efficiency of the *A.incisa* seeds was very low and the lyophilized pure protein was sufficient only to carry out the agglutination assay in *S. cerevisiae*, Trindade had already evaluated the hemagglutinating power of jackin extract and found that it agglutinated mainly erythrocytes from Rabbits and required higher concentrations to agglutinate human erythrocytes, as jackin is 98% homologous to frutackin, it is suggested that frutackin also possesses this hemagglutinating property. The yeasts were supplied by the Central Laboratory of Ceará -LACEN and grown in YEPD culture medium). After culture in Solad brand sheath, model SL183 the yeasts

were washed with ice-cold 0.15M NaCl 5 times. The yeast potato was diluted in 0.15M NaCl until it reached a reading D.O.600nm equal to 0.250 in Allcrom model V-1100 spectrophotometer. The assay was performed in microplates with 50 µL of the sample (2 mg / mL), 50 µL of NaCl and 50 µL of each properly diluted yeast. After 15 min of incubation, the samples were evaluated using a Diag Tech model XJS300 microscope with coupled camera and TSView software (Regente et al., 2014).

13.2 Purification of *Mauritia Flexuosa*

Purification of the chitin ligand lectins from the *Mauritia flexuosa* seeds was carried out at the Biochemistry Laboratory of the Federal University of the São Francisco Valley - UNIVASF, in Petrolina, Pernambuco, Brazil, in the Master's Degree in Natural Resources under the guidance of Professor Wagner Pereira Felix during The month of April 2017

13.2.1 Equipment used:

- Marconi Knife Mill
- LT Vacuum compressor pump LT 65
- Centrifuge DAIKI 80-2B
- Biofilm Filter Syringe Filter Pore Size 0.22mm
- Matrix of Chitin (Sigma-Aldrich);
- Balance BEL engineering
- Glassware
- Wagner type agitator
- UV Spectrophotometer Shimadzu
- Lyophilizer Virtis SP SCIENTIFIC advantage plus

13.2.2 Reagents used

- Dibasic Sodium Phosphate
- Citric Acid
- Buffer Citrate Phosphate 0.1M pH 6.5 with 0.15M NaCl
- Petroleum ether

13.2.3 Origin of Plant Material

The plant material (seeds) of Buriti (*Mauritia flexuosa L.f*). was collected in the urban area of the city of Floriano in the state of Piauí, Brazil, whose coordinates are -6º76'69

"south latitude and -43º02'25" west longitude in April, 2017. A sample of collected material is deposited In the Semiarid Tropic Herbarium (HTSA) in the city of Petrolina (PE) under Number 1,146.

13.2.4 Preparation of the Protein Extract of *Mauritia flexuosa* seeds

Seeds of *Mauritia flexuosa L.f* were crushed in Marconi Knife Mill, and then sifted through 100 mashes resulting in 50g of Buriti flour, the obtained flour was delipidated with Petroleum Ether. After complete delipidation, 450ml of 0.1M Citrate Phosphate Buffer, pH 6.5 with 0.15M NaCl was added in the flour, resulting in a 1:10 dilution, the extract was subjected to stirring on a Wagner agitator type ,overnight . The next day the protein extract was filtered on filter paper twice and centrifuged at 4000g for 30 minutes at room temperature in a DAIKI 80-2B centrifuge. After centrifugation, the supernatant was filtered on a 0.22µM Biofilm Syringe Filter Pore Size filter yielding 375ml of filter protein extract.

Saline Fractionation with Ammonium Sulphate ((NH4)2SO4)

The crude protein extract was subjected to F60 fractionation with Ammonium Sulfate according to the methodology described by Duong-Ly and Gabelli (2014). After dissolution, it was allowed to stand at room temperature for 12 h, then centrifuged at 10,000 g for 30 min at 4°C in a Hettich Zentrifugen, Universal 320R centrifuge. The precipitate was dissolved in water and dialyzed exhaustively against distilled water for 3 days to provide a protein concentrate free of impurities.

13.2.5 Affinity Chromatography in Chitin Column:

Previous studies conducted at the UNIVASF Biochemistry Laboratory have already used affinity chromatography on Agarose-mannose and DEAE-Sephadex A-50 matrices and it was proved that it did not interact with any of these columns, only with

the chitin column, so it was decided that pass the protein extract directly into the chitin column.

The 0.01M Citrate Phosphate Buffer, pH 6.5 with 0.15M NaCl was applied to the chitin column to equilibrate it in gravitational flow, then the Mauritia flexuosa filtered protein extract was applied overnight in the overnight flow , 0.01M Citrate Phosphate Buffer, pH 6.5 with 0.15M NaCl was applied again into the chitin column to equilibrate it. The absorbance was measured in Shimadzu UV Spectrophotometer with reading at 280 nm in quartz cuvette obtaining the following readings

- Buffer: 0.154nm
- Fraction that passed through the chitin column: 0.28nm

Next, Elution with 1M Acetic Acid was started and the reading on Spectrophotometer UV Shimadzu started with the fractions eluted

Later, the fractions that presented the highest reading were collected in Falcon tube and passed to a membrane and dialysed exhaustively against osmosis water at room temperature overnight and then lyophilized in Lytiser Virtis SP SCIENTIFIC advantage plus for 8 hours, obtaining Dried protein samples that were weighed and stored at -20 ° C

13.2.6 Polyacrylamide Gel Electrophoresis under Denaturing Conditions (SDS-PAGE)

To observe the quality and the efficiency of the chromatographic process used for the purification of the protein fraction of the seeds *of M. flexuosa L.f.* And also to determine the apparent molecular mass of the displayed proteins, SDS-PAGE electrophoresis was used to assess its purity, according to Laemmli (1970) with a concentration on the gel of separation of 12%. Were applied 50 µL (2,5 mg / mL) of the

final protein extract and also 5 µL of the Full Range Rainbow ™ Recombinant Protein Molecular Welrer Marker, supplied by Amersham ™.

13.2.7 Determination of the Binding Activity of M. Flexuosa

In order to evaluate whether the protein extract has agglutinating lectins, it is necessary to perform a monitoring assay such as agglutination or hemagglutination assays, Negreiros in 2016 had already verified absence of hemagglutinating activity in the protein extract of *Mauritia flexuosa* in rabbit erythrocytes and of human types A, B, AB and O, then it was chosen to carry out agglutination assay using the yeast *Saccharomyces cerevisiae*, which is a low cost easy growing fungus that has chitin in its cell wall, to confirm if chitin ligand lectins are present. The yeasts were supplied by the Central Laboratory of Ceará - LACEN and grown in YEPD culture plates. After culture in Solad brand sheath, model SL183 the yeasts were washed with ice-cold 0.15M NaCl 5 times. The yeast potato was diluted in 0.15M NaCl until it reached a reading D.O.600nm equal to 0.250 in Allcrom model V-1100 spectrophotometer. The assay was performed in microplates with 50 µL of the sample (2,5 mg / mL), 50 µL of NaCl and 50 µL of each properly diluted yeast. After 15 min of incubation, the samples were evaluated using a Diag Tech model XJS300 microscope with coupled camera and TSView software (Regente et al., 2014).

13.2.8 Evaluation of the Thermal Stability of *M. flexuosa*

The protein extract retained in the chitin column containing the chitin binding lectins was subjected to the thermostability test, it is known that the monocotyledone lectins have more resistance to high temperatures, the extract was subjected to the autoclaving heat treatment (100 °C, 1 kgf.cm-2, 20 min.). After this treatment, 6 mL of the obtained sample was applied on a chitin column following the same conditions previously described, in order to verify if the proteins still maintained their site of

recognition to the carbohydrate and also done agglutination test to evaluate if this still maintained its activity.

13.2.9 : Antifungal assay of M.flexuosa chitin binding lectins in *Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus*

The antifungal tests were carried out at the Central Laboratory of Ceará - LACEN in the period of May 2017 under the authorization of Dr. Liana Perdigão Mello, all reagents, bacterial strains and materials were supplied by the laboratory itself and the experiments were always carried out under supervision Professionals to ensure the quality of the process.

*Candida albicans (*ATCC® 10231™*), Cryptococcus neoformans (*ATCC® 14116™), and *Aspergillus fumigatus (*ATCC® 1022™) ATCC strains previously isolated were again seeded in Saboraud Agar and re-isolated according to the laboratory's internal protocol in order to ensure sufficient pure material for the subsequent experiments. The assay was done in triplicate, three petri dishes were used for each fungus and in each one was added**:**

- **Protein extract 1:** 50 µL extract with chitin binding lectins (5 mg / mL)
- **Protein extract 2:** 50 µL extract with chitin binding lectins (2.5 mg / mL)
- **Protein + Anti Fungal Extract:** 25 µL extract with chitin binding lectins (5 mg / mL) + 25 µL of Anti Fungal Fluconazole (5 mg / mL)
- **Positive Control:** 50 µL of Anti Fungal Fluconazole (5 mg / mL)
- **Negative Control:** 50 µL of saline solution (0.15M NaCl)

14 - Results and discussion

14.1 *Artocarpus incisa* Assays

14.1.1 Frutackin Purification

The Frutackin purification protocol is complex and takes to much time, where first was made the depletion of other more abundant lectins present in the crude extract, for later obtain pure frutackin protein extract after chitin column affinity chromatography. The electronic absorption with 280nm reading of the fruit protein extract revealed 0.6mg / ml of total proteins. A total of 10 purification processes were carried out in which 50g of breadfruit seed flour were used in each process, homogenized in 450mL of buffer (1; 10m / v), obtaining 500mL of crude extract, after elution of the fraction retained in the chromatography chitin column affinity, a protein extract of 10 ml was obtained and after lyophilization only 0.3 mg of lyophilized fruit was obtained for each purification, this purification process was repeated 10 times and the total values of material used and yield of the 5 processes are are in the table below.

Seeds (g)	Flour (g)	Crude Extract (mL)	Protein Extract	Chitin Binding Lectin - Frutackin (mg)
500g	500g	5000mL	100mL	3mg

Table 1 : purification yield of Frutackin

Because of the extremely low purification results, it was only possible to carry out the electrophoresis and the agglutination test in *S.cerevisea*, unfortunately sufficient material was not obtained for the antifungal assays. As the purification process of Frutackin has to be done using fresh seeds to ensure greater stability and better yield, it was decided to use only a batch of 500g of seeds of Fruits harvested on the same day and place, if collected again, the material protein obtained in the first batch could lose its stability, a study by Trindade in protein extract containing jackin that is 98% homologous to frutackin, showed that it also has limited stability in function of the time and storage conditions, therefore to obtain a significant amount of frutackin to perform tests, it would be necessary more columns and investigators performing the purification process simultaneously.

14.1.2 Frutackin Electrophoresis

In SDS-PAGE with tricin of frutackin, the molecular weight marker is at line 1, the superdex entry at line 2, and the protein extract containing other lectins (Frutapin, Frutackin and Frutalin) at line 3, the fruit protein extract pure in streak 4, the various bands in streak 3 indicate other lectins of varied molecular weights, frutackin was eluted around 10mL corresponding to 12kDa of molecular weight, this lectin has an electrophoretic profile very similar to jackin

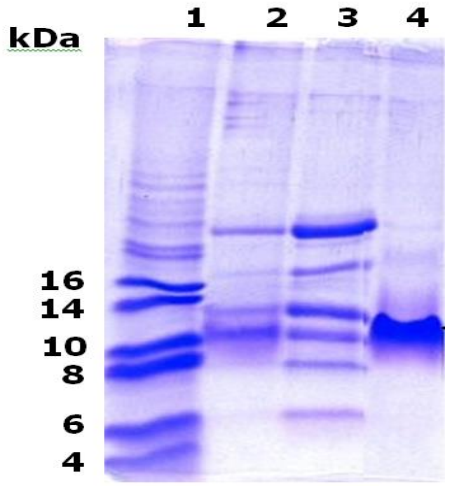


Figure 8: Frutackin Electrophoresis: The electrophoretic profile of frutackin is quite similar to that of jackin, both with a molecular weight of 12 kDa which reinforces the similarity of these lectins, in this way it is possible to predict some biological activities of frutackin from jackin, for example, in a study of purification and structural characterization performed by Trindade in 2005 evaluated some characteristics of jackin, for example, it is unstable at PH lower than 6, so the purification of frutackin was done with 0.1M Citrate Phosphate buffer, pH 6.5 with 0, 15M because of this.

As Frutackin and Jackin are 98% homologous to each other, in terms of molecular mass, secondary structure and primary sequence it is possible to predict some fruitckin functions due to jackin, unfortunately its low yield associated with its low stability as a function of time and storage make it research studies of this lectin more laborious and time-consuming, but it shows a promising potential even in light of its low yield, an alternative would be to employ recombinant DNA Technology to obtain a

recombinant fruit protein in higher yield so as to produce sufficient material for further testing..

Jackin has hemagglutinating activity preferentially in erythrocytes of rabbits in the concentration of 0.3 mg / mL, presenting low haemagglutination in human erythrocytes, which indicates that fruackin also has this hemagglutinating profile due to its 98% structural similarity with jackin, besides the test of inhibition of hemagglutination done by Trindade in 2005 performed with the carbohydrates D-fructose, L-fucose, D-galactose, D-glucose, D-mannose, N-acetyl-galactosamine, N-acetyl-D-glucosamine and N-methyl Showed that none of them was able to inhibit haemagglutination, suggesting that jackin has a preferential affinity for chitin or fragments of chitin, rather than for simple monosaccharides, so probably frutackin has the same characteristics.

Frutackin is a small lectin with only 12kDa, usually small lectins of 10 to 15kDa exhibit good thermal stability, according to Trindade, jackin has thermal stability up to 80 °C, in addition, jackin is able to promote cell adhesion K562 (type of cell line consisting of human erythroleukemia cells, the glycoproteins expressed on the membrane surface of this cell line show that it has glycophorin, found only in erythroid cells present in cells of the human bone marrow) showing to be an important biotechnological study tool and Immunological interaction with tumor cell lines. (Andersson, et al., Trindade 2005).

14.2 *Mauritia flexuosa L.f* assays

14.2.1 Purification of *Mauritia flexuosa L.f* :

Two seeds of Mauritia flexuosa Lf were crushed and sieved resulting in 50g of flour, then 1L of petroleum ether was added for 24h for lipid removal, this process was repeated 7 times, using a total volume of 7L of petroleum ether 7 days after removal of lipids, was diluted in 450 mL of 0.1M citrate phosphate buffer, pH 6.5 with 0.15M NaCl (1: 10m / v), filtered on standard filter paper, centrifuged, filtered again on a membrane filter Of 0.22µm resulting in 375mL of crude extract without solid residues. After fractionation with Ammonium Sulfate (F60) and affinity chromatography on a chitin column, the fraction attached to the chitin column was eluted with 1M acetic acid. The eluted fractions were collected in 1.5mL eppendofrs and read in a spectrophotometer with 280nm absorbance in a Quartz cuvette to measure the fractions that were being eluted, with the objective of identifying the protein peaks according to the chromatogram below.

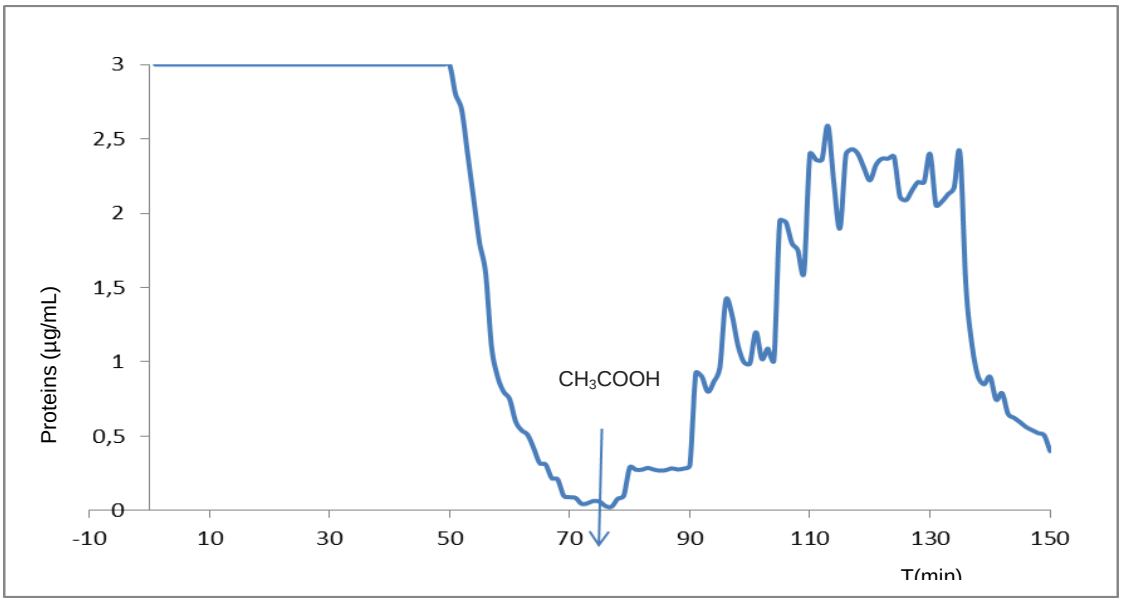


Figure 9: Lectins from *Mauritia flexuosa* chromatogram: the first peak with total proteins absorbance reading above 3 in the first minutes, after elution with CH_3COOH , the reading of chitin binding proteins gave rise to the second protein peak

After reading the spectrophotometer, the fractions with the highest protein value were collected, obtaining 102mL of pure protein extract containing chitin-binding isolecins. From this volume, aliquots of 6mL were assayed for agglutination and a further 6mL were tested for thermostability, resulting in 90mL of extract which will be lyophilized for antifungal and electrophoresis assay. After dialysis of 90mL of the protein extract with osmosis water for 48h for withdrawal of other molecules and lyophilization for 8h, 30mg of pure protein containing only chitin binding lectins were obtained. The purification of *M. flexuosa* showed good extractive yield, where only two units were sufficient to produce 50g of flour, which from its 500ml crude extract (1: 10m / v) obtained 90mL of pure protein extract and 30mg of lyophilized protein Pure, showing a much higher yield than frutackin, according to the table below.

Seeds	Flour (g)	Crude Extract (mL)	Protein Extract	Lectin chitin binding (mg)
2	50g	500mL	90ml	30mg

Table 2: purification yield of Lectins chitin binding from *M.flexuosa*

The purification process is also much simpler, with less cost and with fewer steps, as it has only lectins chitin binders, so the affinity chromatography is done directly on the chitin column, without the need for depletion of other lectins, saving time and reagents, the biggest delay is in the lipid withdrawal process that takes about a week.

14.2.2 Electrophoresis *of Mauritia flexuosa* lectins

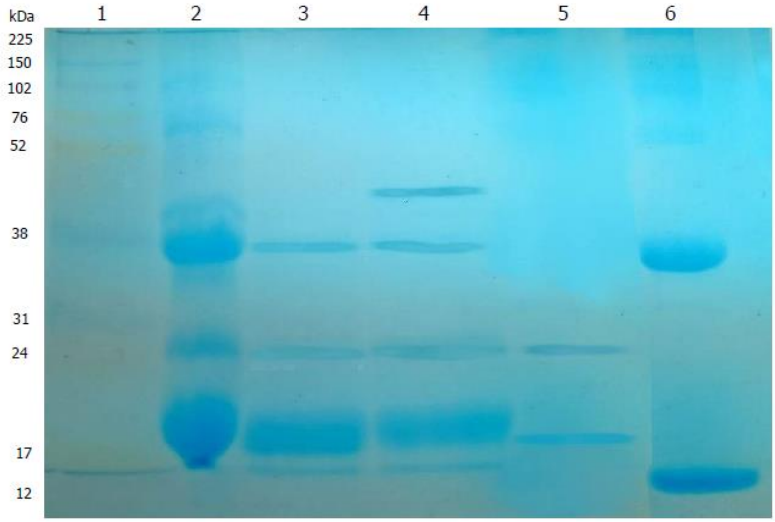


Figure 11: Electrophoresis of *Mauritia flexuosa* lectins: The crude extract showed 3 bands, extract with chitin binding lectins showed 4 bands, and extract with lectin ligands of chitin after autoclaving showed 2 bands. The same results can be observed for the lectin of Arisaema utilie (Dhuna et al) and Caladium bicolor (Kaur et al., 2006), both of the Araceae family, which were characterized by these authors as isolectins. These authors suggest that these lectins have characteristics of isolectins and (or) are charged isomers.

- Well 1: molecular marker
- Well 2: fraction F60
- Well 3: crude extract before entering the chitin column
- Well 4: extract with chitin binding lectins
- Well 5: extract with lectin binders of chitin after autoclaving
- Well 6: BSA and lysozyme

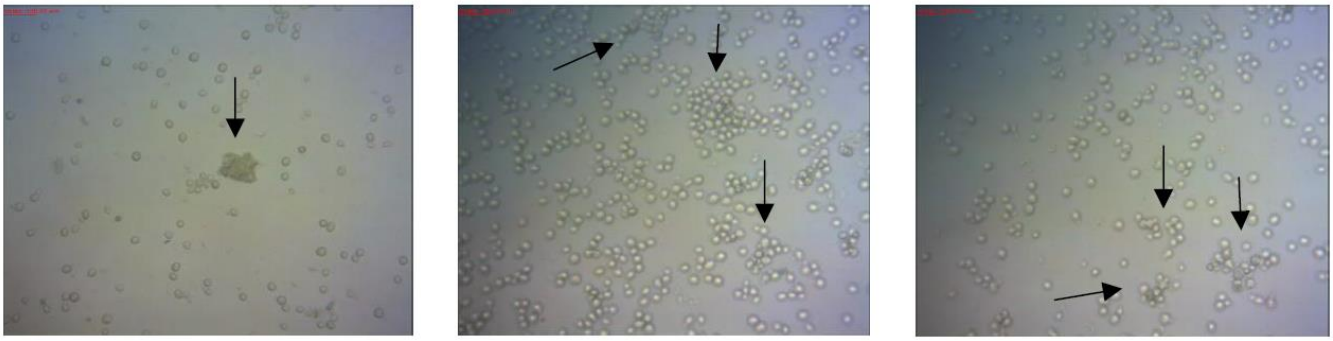
14.2.3 Binding Activity of *M. Flexuosa* assay:

To evaluate whether the protein extract retained in the chitin column had binder activity in fungi, a previous agglutination assay was performed using yeast *Saccharomyces cerevisiae*, which is a low cost easy growing fungus that possesses chitin in its cell wall , to confirm if chitin ligand lectins are present.

The assay was done in triplicate, three positive control plates were incubated with *S. cerevisiae* and 1mL of the protein extract retained in the chitin column eluted with 1M acetic acid was added to each plate, and another three negative control plates were incubated with 1 mL each Of 0.15M NaCl and *S. cerevisiae*, plates were incubated overnight for 12h. We can observe in the sharp agglutination in the plates containing protein extract with lectin ligands of chitin which proves the agglutinating activity of these lectins in fungi with cell wall rich in chitin.

Negreiros in 2016 through the agglutinating activity assay observed that *M. flexuosa* lectins did not exhibit inhibition of agglutinating activity by simple sugars (D-Maltose, D-Lactulose and D-Galactose) which indicates that these lectins may actually have More affinity for chitin.

Positive control: protein extract with chitin binding lectins applied in plates with *S. cerevisiae*



Negative Control with 0.15M NaCl and *S. cerevisiae*

Figure 12: Binding activity of the protein extract retained on the chitin column after elution with 1M acetic acid: 6mL of total volume of the extract were used where 3mL were applied in triplicate (1mL in each plate) in the positive control (lectin chitin ligants + yeast), where clear agglutination could be observed after 12h of incubation, and absence of agglutination in the negative control 0.15M and yeast)

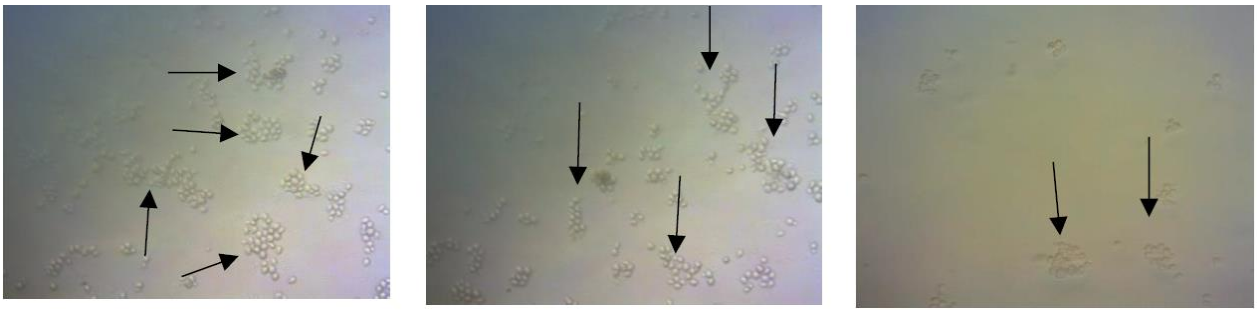
14.2.3 Thermal stability assay:

Proteins normally denature in the presence of high temperatures and lose their biological activity, previous studies in monocotyledon lectins have proved that these are quite stable to heat such as *Setcreasea purpurea* (SPL) lectin, which remained stable up to 80% of its activity (Yao et al., 2010) and *Arisaema utile* lectin (AUL), only lost activity at 80 ° C after 15 min. (Dhuna et al., 2010).

The thermal stability assay of *M. flexuosa* lectin was performed to evaluate whether this lectin maintains its biological activity of binding to chitin present in the chitin column on affinity chromatography and consequently binding to fungal cell walls even after exposure to high temperatures. Then the 6mL aliquot of the total protein extract retained in the column was autoclaved at 100C°, 1 kgf.cm-2, 20 min and then passed back on the chitin column to evaluate whether the lectins present in the extract still had the ability to to bind the chitin present in the column, and thereafter eluted again with 1M acetic acid and performed agglutination test in *S. cerevisiae* in the same manner as in the previous assay.

The proteins retained in the chitin column showed agglutinating activity against *S. cerevisiae* cells even after autoclaving at 100 ° C, 1 kgf.cm-2, 20 min, which proves their high thermal stability even after exposure to high temperature for certain time still maintained its biological activity and presented agglutinating activity in *S. cerevisiae* in addition to the ability to bind to chitin in affinity chromatography, this result was already expected because small lectins of 10 to 15kda are rich in cysteine that covalently bind distant points of the chair polypeptide and increase thermal stability. (Trindade 2005)

Positive control: protein extract retained in the chitin column after auto-claving



Negative Control (0.15M NaCl and S.cereviseae)

Figure 13: Thermostability assay: the first 3 plates that are positive control show clear agglutination even after exposure to autoclaving 100 ° C, 1 kgf.cm-2, 20 min while the negative control containing only yeast + NaCl shows no binding activity.

14.2.4 Anti fungical Assay in *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*

The assay was done in triplicate in each of three fungus, totalizing 9 plates, 3 plates of *Candida albicans (*ATCC® 10231™*),3 plates of Cryptococcus neoformans (*ATCC® 14116™), and 3 plates of *Aspergillus fumigatus (*ATCC® 1022™) where each plate contained

- **Protein extract 1:** 50 µL extract with chitin binding lectins (2.5 mg / mL)
- **Protein extract 2:** 50 µL extract with chitin binding lectins (5 mg / mL)
- **Protein + Anti Fungal Extract:** 25 µL extract with chitin binding lectins (5 mg / mL) + 25 µL of Anti Fungal Fluconazole (5 mg / mL)
- **Positive Control:** 50 µL of Anti Fungal Fluconazole (5 mg / mL)
- **Negative Control:** 50 µL of saline solution (0.15M NaCl)

The 9 plates were incubated at 38 ° C for 72 hours

14.2.4.1 Anti fungical Assay in *Candida albicans*

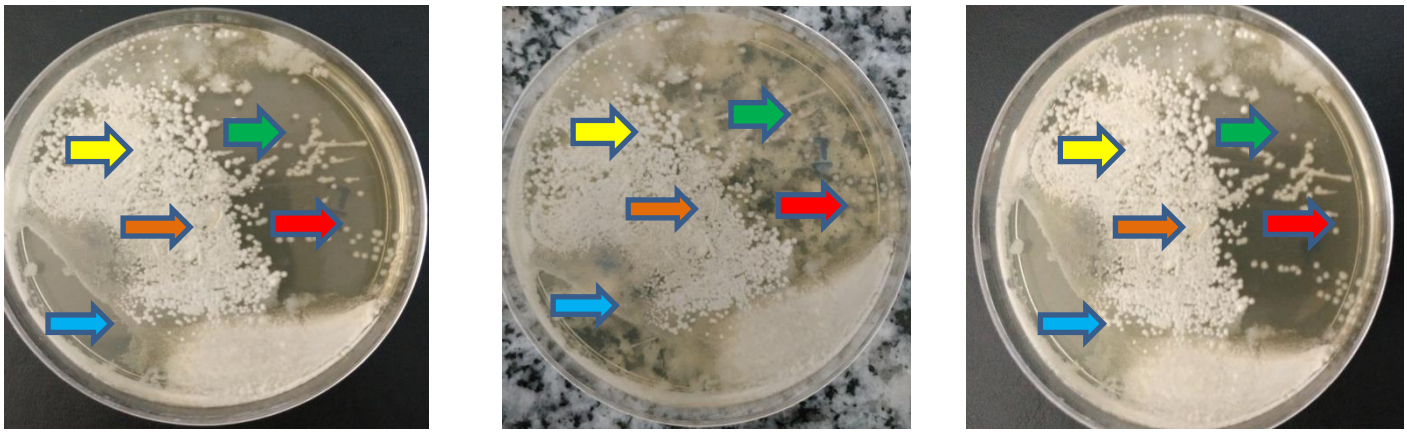


Figure 14: Anti fungal assay in *Candida albicans*

Positive Control (50 µL of Anti Fungal Fluconazole - 5 mg / mL), Protein extract 2 (50 µL extract with chitin binding lectins - 5 mg / mL) and Protein + Anti Fungal Extract (25 µL extract with chitin binding lectins - 5 Mg / mL + 25 µL of Anti Fungal Fluconazole - 5 mg / mL) was able to inhibit *C.albicans* growth on the 3 plates as shown in Picture 12. Protein extract 1(50 µL extract with chitin binding lectins - 2.5 mg / mL)) was not able to inhibit fungal growth, so the minimum inhibitory concentration (MIC) of the lectins to prevent the growth of C. albicans is 5mg / mL, it was also shown that the lectin (5mg / mL) associated with antifungal (Fluconazole) exerts agonist inhibitory effect, in this way the lectin can serve as molecular target of binding to the cell wall rich in chitin of fungi

- Protein extract 1: 50 µL extract with chitin binding lectins (2.5 mg / mL)
- Protein extract 2: 50 µL extract with chitin binding lectins (5 mg / mL)
- Protein + Anti Fungal Extract: 25 µL extract with chitin binding lectins (5 mg / mL) + 25 µL of Anti Fungal Fluconazole (5 mg / mL)
- Positive Control: 50 µL of Anti Fungal Fluconazole (5 mg / mL)
- Negative Control: 50 µL of saline solution (0.15M NaCl)

14.2.4.2 Anti fungical Assay in *Aspergillus fumigatus*

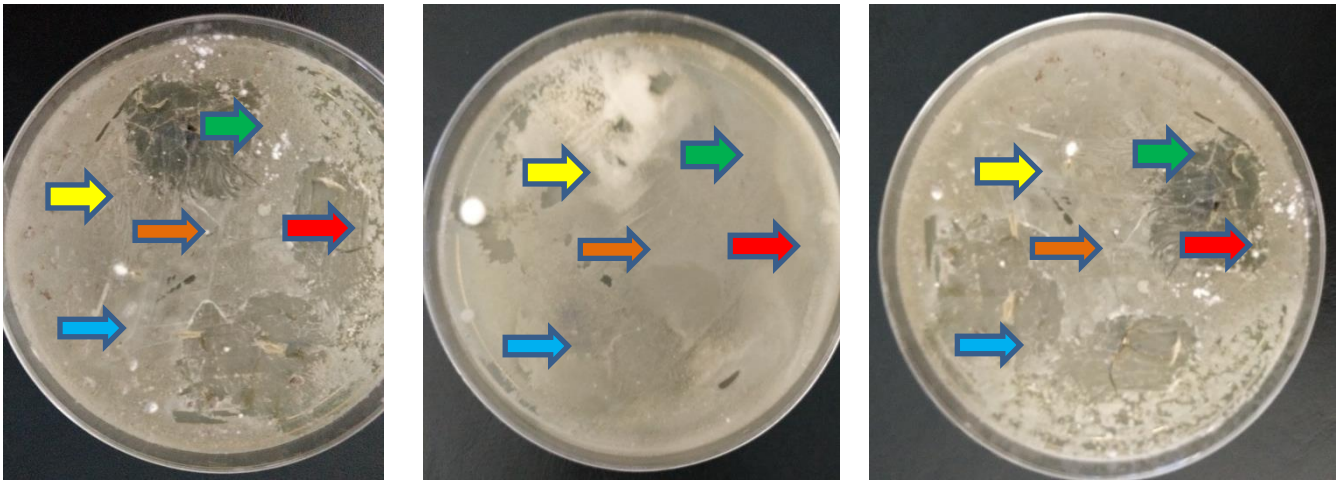


Figure 15 : Anti fungical Assay in *Aspergillus fumigatus*

The antifungal assay did not obtain satisfactory results in A*spergilus fumitatus*, where neither chitin binding lectins nor the positive control were able to inhibit this fungus, in this way it is a fungus resistant to these lectins.

Protein extract 1: 50 µL extract with chitin binding lectins (2.5 mg / mL)

Protein extract 2: 50 µL extract with chitin binding lectins (5 mg / mL)

Protein + Anti Fungal Extract: 25 µL extract with chitin binding lectins (5 mg / mL) + 25 µL of Anti Fungal Fluconazole (5 mg / mL)

Positive Control: 50 µL of Anti Fungal Fluconazole (5 mg / mL)

Negative Control: 50 µL of saline solution (0.15M NaCl)

14.2.4.3 Anti fungical Assay in *Cryptococcus neoformans*

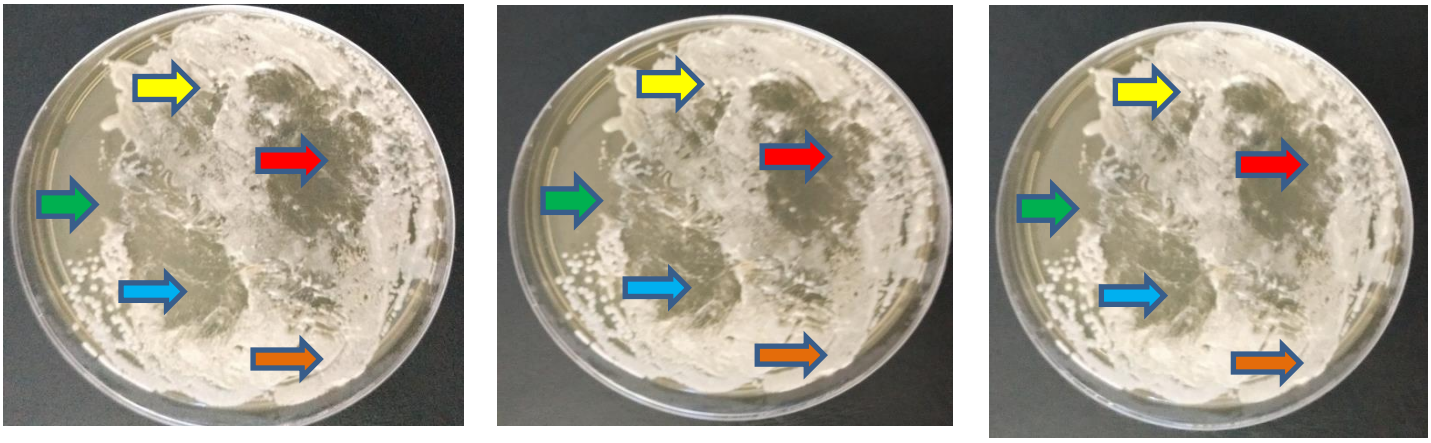


Figure 16: Anti fungical Assay in *Cryptococcus neoformans*

Positive Control (50 µL of Anti Fungal Fluconazole - 5 mg / mL), Protein extract 2 (50 µL extract with chitin binding lectins - 5 mg / mL) and Protein + Anti Fungal Extract (25 µL extract with chitin binding lectins - 5 Mg / mL + 25 µL of Anti Fungal Fluconazole - 5 mg / mL) was able to inhibit *Cryptococcus neoformans* growth on the 3 plates as shown in Picture 14. Protein extract 1(50 µL extract with chitin binding lectins - 2.5 mg / mL)) was not able to inhibit fungal growth, so the minimum inhibitory concentration (MIC) of the lectins to prevent the growth of *Cryptococcus neoformans* is 5mg / mL, it was also shown that the lectin (5mg / mL) associated with antifungal (Fluconazole) exerts agonist inhibitory effect, in this way the lectin can serve as molecular target of binding to the cell wall rich in chitin of fungi

- Protein extract 1: 50 µL extract with chitin binding lectins (2.5 mg / mL)
- Protein extract 2: 50 µL extract with chitin binding lectins (5 mg / mL)
- Protein + Anti Fungal Extract: 25 µL extract with chitin binding lectins (5 mg / mL) + 25 µL of Anti Fungal Fluconazole (5 mg / mL)
- Positive Control: 50 µL of Anti Fungal Fluconazole (5 mg / mL)
- Negative Control: 50 µL of saline solution (0.15M NaCl)

15 – CONCLUSION

The chitin binding lectins are a promising alternative for the development of anti fungal drugs with fewer side effects and more selective molecular target. Frutackin purified from *A.incisa* has a very low yield but exhibits antifungal potential, since it has been able to agglutinate *S.cereviseae*, besides it has 98% structural similarity with Jjackin which is a lectin already studied and characterized having several scientific applications. We suggest the possibility of research studies to use recombinant DNA technology to produce recombinant proteins on a large scale.

The purified lectins of *M. flexuosa* are a more affordable and low cost option, with good yield, high resistance to high temperatures and satisfactory antifungal potential, besides not agglutinating human erythrocytes, in this way, probably presents security to be incorporated in forms topical or oral use. Further studies will be needed to better characterize the lectins of *M. flexuosa* in addition to its efficacy.

Further studies are needed to prove the safety of these lectins and to evaluate the best pharmaceutical form for incorporation besides other possible biological activities. For the production of new drugs for oral use, we can apply nanoparticles of galactomannans and xyloglucans to controlled release drugs to ensure improvement of pharmacokinetics and pharmacodynamics, and for the development of ointments can be applied gels based on plant hemicelluloses containing D-galactose binding lectins for modulation of the cicatricial process and improving lesions caused by fungi associated with the binding power of the chitin binding lectins.

16 – BIBLIOGRAPHY

"Artocarpus altilis". Germplasm Resources Information Network. United States Department of Agriculture. 2007-07-03. Retrieved 2009-02-21.

Alpuche, J; Pereyra, A; Agundis, C; Rosas, C; Pascual, C; Slomianny, M-C; Vázquez, L; Zenteno, E. Purification and characterization of a lectin from the white shrimp Litopenaeus setiferus (Crustacea decapoda) hemolymph. Biochimica et Biophysica Acta (BBA) - General Subjects, v. 1724, p. 86-93, 2005.

Ambrosio, AL; Sanz, L; Sánchez, EI; Wolfenstein-Todel, C; Calvete, JJ. Isolation of two novel mannan- and L-fucose-binding lectins from the alga Enteromorpha prolifera: biochemical characterization of EPL-2. Archives of Biochemistry and Biophysics, v. 415, p. 245-50, 2003.

Andrade, CAS; Correia, MTS; Coelho, LCBB; Nascimento, SC; Santos-Magalhães, NS. Antitumor activity of Cratylia mollis lectin encapsulated into liposomes. International Journal of Pharmaceutics, v. 278, p. 435-45, 2004.

Araujo R, Rodrigues AG. Variability of germinative potential among pathogenic species of Aspergillus. J Clin Microbiol 2004; 42:4335-7.

Arendash GW, Jensen MT, Salem Jr. N, Hussein N, Cracchiolo J, Dickson A, et al. A died high in omega-3 fatty acids does not improve or protect cognitive performance in Alzheimer's transgenic mice. Neuroscience 2007;149:286–302.

Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D, Lortholary O, Maertens J, Meis JF, Patterson TF, Ritter J, Selleslag D, Shah PM, Stevens DA, Walsh TJ; Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002; 34:7-14.

Babal, P; Russell, LC. Sialic acid-specific lectin-mediated adhesion of Tritrichomonas foetus and Tritrichomonas mobilensis. The Journal of Parasitology, v. 85, p. 33-40, 1999.

Baddour LM, Perfect JR, Ostrosky-Zechener L. Successful use of amphotericin B Balzarini, J. Inhibition of HIV entry by carbohydrate-binding proteins. Antiviral Research, v. 71, p. 237-47, 2006.

Bandyopadhyay, S; Roy, A; Das, S. Binding of garlic (Allium sativum) leaf lectin to the gut receptors of homopteran pests is correlated to its insecticidal activity. Plant Science, v. 161, p. 1025–33, 2001.

Banerjee, S; Chaki, S; Bhowal, J; Chatterjee, BP. Mucin binding mitogenic lectin from freshwater Indian Belamyia bengalensis: purification and molecular characterization. Archives of Biochemistry and Biophysics, v. 421, p. 125-34, 2004.

Barre, A; Peumans, WJ; Rossignol, M; Borderies, G; Culerrier, R; Van Damme, EJM; Rougé, P. Artocarpin is a polyspecific jacalin-related lectin with a monosaccharide preference for mannose. Biochimie, v. 86, p. 685-91, 2004.

Bazil, V; Entlicher, G. Complexity of lectins from the hard roe of perch (Perca fluviatilis L.). The International Journal of Biochemistry & Cell Biology, v. 31, p. 431-42, 1999.

Bennett JE, Dismukes WE, Duma RJ, Medoff G, Sande MA, Gallis H, Leonard J, Benson CA, Kaplan JE, Masur H, Pau A, Holmes KK, CDC - Centers for Disease Control; National Institutes of Health; Infectious Diseases Society of America. Treating opportunistic infections among HIV infected adults and adolescents. MMWR -Morbidity and Mortality Weekly Report 53 (RR15): 1-112, 2004.

Bertrand, O; Cochet, S; Cartron, J-P. Expanded bed chromatography for one-step purification of mannose binding lectin from tulip bulbs using mannose immobilized on DEAE Streamline. Journal of Chromatography A, v. 822, p. 19-28, 1998.

Bicanic T, Harrison T, Niepieklo A, Nontobeko D, Meintjes G. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazol monotherapy: the role of fluconazole resistance and immune reconstitution. Clinical of Infectious Diseases 43: 1069-1073, 2006.

Bicanic T, Wood R, Meintjes Grebe,K, Brower A, Loyse A, Bekker LG, Jaffar S, Harrison T. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. Clinical of Infectious Diseases 47:123-130, 2008.

Bouwman, LH; Roep, BO; Roos, A. Mannose-Binding Lectin: Clinical Implications for Infection, Transplantation, and Autoimmunity. Human Immunology, v. 67, p. 247-56, 2006.

Cammarata, M., Benenati, G., Odom, E. W., Salerno, G., Vizzini, A., Vasta,G. R. & Parrinello, N. (2007). Isolation and characterization of a fish F-type lectinfrom gilt head bream (Sparus aurata) serum. Biochimica et Biophysica Acta, Vol.1770, No. 1, (January 2007), pp. 150-155, 0304-4165.

Campisi G, Pizzo G, Milici ME. Candidal carriage in the oral cavity of human immunodeficiency virus- infected subjects. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;93:281-6.

Chamilos G, Kontoyiannis DP. Defining the diagnosis of invasive aspergillosis. Med Mycol 2006; 44:S163-72.

Chang S-T, Miles PG. (2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact. [S.l.]: CRC Press. ISBN 0849310431.

Che, A.-F., Huang, X.-J. & Xu, Z.-K. (2011). Polyacrylonitrile-basednanofibrous membrane with glycosylated surface for lectin affinity adsorption.Journal of Membrane Science, Vol. 366, No. 1-2, (January 2011), pp. 272-277,0376-7388.

Chu, KT; Ng, TB. Smilaxin, a novel protein with immunostimulatory, antiproliferative, and HIV-1-reverse transcriptase inhibitory activities from fresh Smilax glabra rhizomes. Biochemical and Biophysical Research Communications, v. 340, p. 118-24, 2006.

Coelho, M. B.; Marangoni, S.; Macedo, M. L. R.. Insecticidal action of Annona coriacea lectin against the flour moth Anagasta kuehniella and the rice moth Corcyra cephalonica (Lepidoptera: Pyralidae). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, v. 146, n. 3, p. 406-414, 2007.

Cooke M.C . Fungi: Their Nature and Uses. M. J. Berkeley. Correia J.L.A Purification, Partial Characterization And Potentiality Iotechnology Of Three Lectins Of Seeds Of Species Of Leguminosae Of The Subtribo Diocleinae.. Doctoral Thesis Submitted To The Coordination Of The Postgraduate Course In Biotechnology Of The Federal University Of Ceará. 2015.

Correia, M. T. S.; Coelho, L. C. B. B.; Paiva, P. M. G. Lectins, carbohydrate recognition molecules: are they toxic?. In: Yasir Hasan Siddique. (Org.). Recent Trends in Toxicology Kerala, India: Transworld Research Network, v. 37, p. 47-59. 2008.

Coutiño-Rodríguez, R.; Hernández-Cruz, P.; Giles-Ríos, H. Lectins in fruits having gastrointestinal activity: Their participation in the hemagglutinating property of Escherichia coli 0157:H7. Archives of Medical Research, v. 32, p. 251-257, 2001.

De Mejía EG & Prisecaru VI. Lectins as bioactive plant proteins: a potential in cancer treatment. Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Chicago. 2005.

Delatorre, P. et al.. Crystal structure of a lectin from Canavalia maritima (ConM) bin complex with trehalose and maltose reveals relevant mutation in ConA-like lectins. Journal of Structural Biology v. 154, p. 280–286, 2006.

Demain AL, Fang A. (2000). «The natural functions of secondary metabolites». Advances in Biochemical Engineering/Biotechnology. 69: 1–39. doi:10.1007/3-540-44964-7_1. PMID 11036689.

Desjardin DE, Oliveira AG, Stevani CV. (2008). «Fungi bioluminescence revisited». Photochemical & Photobiological Sciences. 7 (2): 170–82. doi:10.1039/b713328f. PMID 18264584.

Dhuna, V.; Dhuna, K.; Singh, J.; Saxena, A. K.; Agrawal, S. K.; Kamboj, S. S. Isolation, purification and characterization of an N-acetyl-D-lactosamine binding mitogenic and anti-proliferative lectina from tubers of a cobra lily Arisaema utile Schott. Advances in Bioscience and Biotechnology. v. 1, p. 79-90, 2010.

Dunphy, JL; Barcham, GJ; Bischof, RJ; Young, AR; Nash, A; Meeusen, ENT. Isolation and characterization of a novel eosinophyl-specific galectin released into the lungs in response to allergen challenge. The Journal of Biological Chemistry, v. 277, p. 14916-24, 2002.

Elifio, SL; Silva, MLCC; Iacomini, M; Gorin, PAJ. A Lectin from the lichenized Basidiomycete Dictyonema glabratum. New Phytologist, v. 148, p. 327-34, 2000.

Endress, B. A., Horn, C. M., Gilmore, M. P. Mauritia flexuosa palm swamps: composition, structure and implications for conservation and management. For. Ecol. Manag. v. 302, p. 346–353, 2013.

Erika L. PfaunmillerMarie Laura PaulemondCourtney M. DupperDavid S. Hage. Affinity monolith chromatography: a review of principles and recent analytical applications. Analytical and Bioanalytical Chemistry March 2013, Volume 405, Issue 7, pp 2133–2145.

Fernández-Alonso, J.L et al. Lectin prospecting in Colombian Labiatae. A systematicecological approach. Biochemical Systematics and Ecology, v. 31, n. 6, p. 617-633, 2003.

Fields BT, Bradshaw M, Haywood H, McGee ZA, Cate TR, Cobbs CG, Warner JF,
Freire, M. G. M. Isolation and partial characterization of a novel lectin from Talisia esculenta seeds that interferes with fungal growth. Plant Physiology and Biochemistry v.40, p. 61-68, 2002.
Gabor, F.L. The lectin-cell interaction and its implications to intestinal lectin-mediated drug delivery. Advanced Drug Delivery Reviews v. 56, n.4, p. 459-480, 2004.

Garcia-Pino, A; Buts, L; Wyns, L; Loris, R. Interplay betwwen metal binding and cis/trans isomerization in legume lectins: structural and thermodynamic study of P. angolensis lectin. Journal of Molecular Biology, v. 361, p. 153-67, 2006.

Gavrovic-Jankulovic, M; Poulsen, K; Brckalo, T; Bobic, S; Lindner, B; Petersen, A. A novel recombinantly produced banana lectin isoform is a valuable tool for glycoproteomics and a potent modulator of the proliferation response in CD3+, CD4+, and CD8+ populations of human PBMCs. The International Journal of Biochemistry & Cell Biology, v. 40, p. 929-41, 2008.

Goldstein, IJ; Winter, HC; Aurandt, J; Confer, L; Adamson, JT; Hakansson, K; Remmer, H. A new α-galactosyl-binding protein from the mushroom Lyophyllum decastes. Archives of Biochemistry and Biophysics, v. 467, p. 268-74, 2007.

Gregory, N. Special patient populations: Onychomycosis in the HIV-positive patient. J Am Acad Dermatol 1996:35(3):13-16.

Gualco L, Debbia EA, Bandettini R, Pescetto L, Cavallero A, Ossi MC, et al. Antifungal resistance in Candida spp isolated in Italy between 2002 and 2005 from children and adults. International Journal of Antimicrobial Agents 2007:29: 179-84.

Guardia, Anna; Lozano, Francisco ; Mannose-binding lectin deficiencies in infectious and inflammatory disorders. Reviews in Medical Microbiology: April 2003 - Volume 14 - Issue 2 - pp 41-52 Immunology.

Gupta, N; Srivastava, PS. Purification and characterization of a lectin from seeds and cotyledonary callus of Zizyphus mauritiana. Plant Cell Reports, v. 17, p. 552-6, 1998.
Harris SD. (2008). «Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems». Mycologia. 50 (6): 823–32. doi:10.3852/08-177. PMID 19202837.

Hartley, MR; Lord, JM. Cytotoxic ribosome-inactivating lectins from plants. Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics, v. 1701, p. 1-14, 2004.

Hawksworth DL. (2006). «The fungal dimension of biodiversity: magnitude, significance, and conservation». Mycological Research. 95: 641–55. doi:10.1016/S0953-7562(09)80810-1.

Hibbett DS; (2007). «A higher level phylogenetic classification of the Fungi» (PDF). Mycological Research. 111 (5): 509–47. doi:10.1016/j.mycres.2007.03.004.

Howard RJ, Ferrari MA, Roach DH, Money NP. (1991). «Penetration of hard substrates by a fungus employing enormous turgor pressures». Proceedings of the National Academy of Sciences USA. 88 (24): 11281–84. doi:10.1073/pnas.88.24.11281. PMC 53118Acessível livremente. PMID 1837147.

Imbert, A. et al. Structures of the lectins from Pseudomonas aeruginosa: insights into themolecular basis for host glycan recognition. Microbes and Infection, v. 6, n. 2, p. 221-228,2004.

Ishikawa T, Zhu B-L, Li D-R, Zhao D, Michiue T, Maeda H. Age-dependent increase of clusterin in the human pituitary gland. Legal Med 2006;8:28-33.

Jiang, J-F; Han, Y; Xing, L-J; Xu, Y-Y; Xu, Z-H; Chong, K. Cloning and expression of a novel cDNA encoding a mannose-specific jacalin-related lectin from Oryza sativa. Toxicon, v. 47, p. 133-9, 2006.

John T. Arnason, Rachel Mata, John T. Romeo. Phytochemistry of Medicinal Plants. Series Volume 29, Copyright 1995 Publisher Springer US Copyright Holder SpringerScience+Business Media New York. Number of Pages VIII, 364. Edition Number 1. eBook ISBN 978-1-4899-1778-2 DOI 10.1007/978-1-4899-1778-2.

Jung, EC; Kim, KD; Bae, CH; Kim, JC; Kim, DK; Kim, HH. A mushroom lectin from ascomycete Cordyceps militaris. Biochimica et Biophysica Acta, v. 1770, p. 833–8, 2007.

Kanazawa, N. Dendritic cell immunoreceptors: C-type lectin receptors for pattern-recognition and signaling on antigen-presenting cells. Journal of Dermatological Science, v. 45, p. 77-86, 2007.
Kanazawa, N; Tashiro, K; Miyachi, Y. Signaling and immune regulatory role of the dendritic cell immunoreceptor (DCIR) family lectins: DCIR, DCAR, dectin-2 and BDCA-2. Immunobiology, v. 209, p. 179-90, 2004.

Karasaki, Y; Tsukamoto, S; Mizusaki, K; Sugiura, T; Gotoh, S. A garlic lectin exerted an antitumor activity and induced apoptosis in human tumor cells. Food Research International, v. 34, p. 7-13, 2001.

Kaur, A; Singh, J; Kamboj, SS; Sexana, AK; Pandita, RM; Shamnugavel, M. Isolation of an N-acetyl-d-glucosamine specific lectin from the rhizomes of Arundo donax with antiproliferative activity. Phytochemistry, v. 66, p. 1933-40, 2005.

Kaur, M; Singh, K; Rup, PJ; Saxena, AK; Khan, RH; Ashraf, MT; Kamboj, SS; Singh, J. A tuber lectin from Arisaema helleborifolium Schott with anti-insect activity against melon fruit fly, Bactrocera cucurbitae (Coquillett) and anti-cancer effect on human cancer cell lines. Archives of Biochemistry and Biophysics, v. 445, p. 156-65, 2006.

Keller NP, Turner G, Bennett JW. (2005). «Fungal secondary metabolism – from biochemistry to genomics». Nature Reviews in Microbiology. 3 (12): 937–47. doi:10.1038/nrmicro1286. PMID 16322742.

Keller NP, Turner G, Bennett JW. (2005). «Fungal secondary metabolism—from biochemistry to genomics». Nature Reviews Microbiology. 3 (12): 937–47. doi:10.1038/nrmicro1286. PMID 16322742

Kennedy, J. F. et al. Lectins, versatile proteins of recognition: a review. Carbohydrate Polymers, v. 26, n. 3, p. 219-30, 1995.

Kishore, U; Greenhough, TJ; Waters, P; Shrive, AK; Ghai, R; Kamran, MF; Bernal, AL; Reid, KBM; Madan, T; Chakraborty, T. Surfactant proteins SP-A and SP-D: Structure, function and receptors. Molecular Immunology, v. 43, p. 1293-315, 2006.

Koike, T; Beppu, H; Kuzuya, H; Maruta, K; Shimpo, K; Suzuki, M; Titani, K; Fujita, K. A 35 kDa mannose-binding lectin with hemagglutinating and mitogenic activities from “Kidachi Aloe” (Aloe arborescens Miller var. natalensis Berger). The Journal of Biochemistry, v.118, p. 1205-10, 1995
Konemam, E.W.; Roberts, G.D. - Micologia. Practica de laboratório . 3 ed. Buenos Aires, Panamericana, 1992.

Konozy, EHE; Bernardes, ES; Rosa, C; Faca, V; Greene, LJ; Ward, RJ. Isolation, purification, and physicochemical characterization of a D-galactose-binding lectin from seeds of Erythrina speciosa. Archives of Biochemistry and Biophysics, v. 410, p. 222-29, 2003.

Kurokawa CS, Sugizaki MF, Peraçoli MTS Virulence factors in fungi of systemic mycoses Revista do Instituto de Medicina Tropical São Paulo 40:125-135, 1998. 50.

Kvennefors, E. C. E., Leggat, W., Hoegh-Guldberg, O., Degnan, B. M. & Barnes, A. C. (2008). An ancient and variable mannose-binding lectin from the coral Acropora millepora binds both pathogens and symbionts. Developmental and Comparative Immunology, Vol. 32, No. 12, pp. 1582-1592, 0145-305X.

Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of Cryptococcus neoformans. American Journal of Epidemiology 120:123-130, 1984.

Leite, Y .F. M. M. et al. Purification of a lectin from the marine red alga Gracilaria ornata and its effect on the development of the cowpea weevil Callosobruchus maculatus (Coleoptera: Bruchidae). Biochimica et Biophysica Acta, v. 1724, p. 137-145, 2005.

Leite, YFMM; Silva, LMCM; Amorim, RCN; Freire, EA; Jorge, DMM; Grangeiro, T.B; Benevides, NMB. Purification of a lectin from the marine red alga Gracilaria ornata and its effect on the development of the cowpea weevil Callosobruchus maculates (Coleoptera: Bruchidae). Biochimica et Biophysica Acta, v. 1724, p. 137-45, 2005.

Lerivray, H; Chesnel, A; Jego, P. Identification and localization of lectin in the oviduct of various urodele amphibians. Comparative Biochemistry and Physiology, v. 81B, p. 385-91, 1985.

LI, Y. R. . A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom Pleurotus citrinopileatus. Biochimica et Biophysica Acta, in press, 2007.
lipid complex in the treatment of cryptococcosis. Clinical of Infectious Diseases 40 (suppl 6): 409-413, 2005.

Lis, H.; Sharon, N. Lectins in higher plants. In: MARCUS, A. The Biochemistry of Plants, a Comprehensive Treatise. Proteins and nucleic acids. v.6 New York: Academic Press, p. 371- 447, 1981.

Liu, Z.L., Goh, S.H., Ho, S.H., 2007. Screening of Chinese medicinal herbs for bioactivity against Sitophilus zeamais Motschulsky and Tribolium castaneum (Herbst). J. Stored. Prod. Res. 43, 290-296.

Lorenzi, H., Noblick, LR, KAHN, F., Ferreira, E. Flora Brazilian Lorenzi:. Arecaceae (Palmeiras) Plantarum, Nova Odessa. 2010.

M.W Turner The role of mannose-binding lectin in health and disease Molecular Immunology, 2003, pp. 423-429.

MacDougall L, Kidd SE, Galanis E; et al. (2007). «Spread of Cryptoccus gattii in British Columbia, Canada and detection in the Pacific Northwest, USA». Emerg Infect Dis. 13: 42–50. doi:10.3201/eid1301.060827.

Macedo, M. L. R. et al. Insecticidal action of Bauhinia monandra leaf lectin (BmoLL) against Anagasta kuehniella (Lepidoptera: Pyralidae), Zabrotes subfasciatus and Callosobruchus maculatus (Coleoptera: Bruchidae). Comparative Biochemistry and Physiology A, v. 146, n. 4, p. 486-498, 2007.

Machuka, JS; Okeola, OG; Chrispeels, MJ; Jackai, LEN. The African yam bean seed lectin affects the development of the cowpea weevil but does not affect the development of larvae of the legume pod borer. Phytochemistry, v. 53, p. 667-74, 2000.

Maciel, E. V. M. et al.. Mitogenic activity of Cratylia mollis lectin on human lymphocytes. Biologicals v. 32, n. 1, p. 57-60, 2004.

Magliani W, Conti S, Arseni S, Salati A, Ravanetti L, Maffei DL, Giovati L, Polonelli L. Antibody-mediated protective immunity in fungal infections. New Microbiology 28:299-309, 2005.

Matsumoto, R., Shibata, T. F., Kohtsuka, H., Sekifuji, M., Sugii, N., Nakajima, H., Kojima, N., Fujii, Y., Kawsar, S. M. A., Yasumitsu, H., Hamako, J., Matsui, T. & Ozeki, Y. (2011). 68 Affinity Chromatography Glycomics of a novel type-2 N-acetyllactosamine-specific lectin purified from the feather star, Oxycomanthus japonicus (Pelmatozoa: Crinoidea). Comparative Biochemistry and Physiology, Part B, Vol. 158, No. 4, (April 2011), pp. 266-273, 1096-4959.

Mazneikova V. Vaginal candidiasis – treatment protocols using miconazole and fluconazole. [Review] Akush Ginekol (Sofiia) 2003; 42 (Suppl 2):30-4.

Medina-Flores R, Wang G, Bissel SJ, Murphey-Corb M, Wileya CA. Destruction of extracellular matrix proteoglycans is pervasive in simian retroviral neuroinfection. Neurobiol Dis 2004;16:604-16.

Molchanova, V; Chikalovets, I; Chernikov, O; Belogortseva, N; Li, W; Wang, J-H; Yang, D- YO; Zheng, Y-T; Lukyanov, P. A new lectin from the sea worm Serpula vermicularis: Isolation, characterization and anti-HIV activity. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, v. 145, p. 184-93, 2007.

Molina, MC; Vicente, C. Purification and characterization of two isolectins with arginase activity from the lichen Xanthoria parietina. Journal of Biochemistry and Molecular Biology, v. 33, p. 300-7, 2000.

Monteiro-Moreira, Ana Cristina De Oliveira (2002).Structural Characterization of Three Lectins Presenting Specificities for Different Sugar, Isolated from Fruit Seeds-Bread (Artocarpus incisa L.). Fortaleza, 117p. Thesis (PhD) Federal University of Ceará.

Moreira, R.A.; Ainouz, I.L. Lectins from seeds of jackfruit (Artocarpus integrifolia): isolation and purification of two isolectins from the albumin fractions. Biol. Plant., 23(3):186-192, 1981.

Moreira, RA; Cavada, BS; Oliveira, JTA; Ainouz, IL. Plant lectins. In: Oliveira, B. & Sgarbieri, V. Proceedings of the First Brazilian Congress on Proteins. Campinas, SP: Editora da UNICAMP. p. 71-96. 1990.

Moura, R. M. et al. CvL, a lectin from the marine sponge Cliona varians: Isolation, charactrization and its effects on pathogenic bacteria and Leishmania promastigotes. Comparative Biochemistry and Physiology A, v. 145, n. 4, p. 517-523, 2006.

Moura, RM; Queiroz, AFS; Fook, JMSLL; Dias, ASF; Monteiro, NKV; Ribeiro, JKC; Moura, GEDD; Macedo, LLP; Santos, EA; Sales, MP. CvL, a lectin from the marine sponge Cliona varians: Isolation, characterization and its effects on pathogenic bacteria and Leishmania promastigotes. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, v. 145, p. 517-23, 2006.

Mueller GM, Schmit JP. (2006). «Fungal biodiversity: what do we know? What can we predict?». Biodiversity and Conservation. 16: 1–5. doi:10.1007/s10531-006-9117-7.
MW Douglas, CM Parsons, and T Hymowitz. Nutritional evaluation of lectin-free soybeans for poultry Poultry Science (1999) 78 (1): 91-95

Naeem, A; Khan, RH; Vikram, H; Akif, M. Purification of Cajanus cajan root lectin and its interaction with rhizobial lipopolysaccharide as studied by different spectroscopic techniques. Archives of Biochemistry and Biophysics, v. 396, p. 99-105, 2001.

Negreiros, Crp ; Cruz, Drr ; Wedge , Mc; France, Rsrl ; Felix, Wp. Purification and characterization PARTIAL LECTIN Mauritia SEED thermostable flexuosa Lf binder Saccharomyces cerevisiae. Laboratory of Biochemistry (CCA) - UNIVASF, Petrolina, PE. 2 Center of Biological Sciences Programme Master Degree in Biological Sciences, UFPE, Recife, PE.

Neila C. A. Ximenes. Insecticidal activity of Caesalpinia ferrea pod lectin on Nasutitermes corniger (Isoptera: Termitidae) 2009).

Neumann D. et al. Computational modeling of the sugar-lectin interaction. Advanced Drumorg Delivery Reviews, v. 56, n. 4, p. 437-457, 2004.

Ngai, PHK; Ng, TB. A mushroom (Ganoderma capense) lectin with spectacular thermostability, potent mitogenic activity on splenocytes, and antiproliferative activity toward tumor cells. Biochemical and Biophysical Research Communications, v. 314, p. 988-93, 2004.

Nomura, K; Ashida, H; Uemura, N; Kushibe, S; Ozaki, T; Yoshida, M. Purification and characterization of a mannose/glucose-specific lectin from Castanea crenata. Phytochemistry, v. 49, p. 667-73, 1998.

olnick JR. Oral candidosis. Report of case implicating Candida parapsilosis as a pathogen. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics, 1980:50(5): 411-15.

Ooi, LSM; Sun, SSM; Ooi, VEC. Purification and characterization of a new antiviral protein from the leaves of Pandanus amaryllifolius (Pandanaceae). The International Journal of Biochemistry & Cell Biology, v. 36, p. 1440-6, 2004.

Ordóñez R. M. et al. Antimicrobial activity of glycosidase inhibitory protein isolated from Cyphomandra betacea Sendt. fruit. Peptides, v. 27, p.1187-1191, 2006.

Ourth, DD; Narra, MB; Chung, KT. Isolation of mannose-binding C-type lectin from Heliothis virescens pupae. Biochemical and Biophysical Research Communications, v. 335, p. 1085-9, 2005.

Paiva, P. M. G.,l. Purification and primary structure determination of two Bowman-Birk type trypsin isoinhibitors from Cratylia mollis seeds. Phytochemistry, v. 67, n. 6, p. 545-552, 2006.

Parisi, MG; Moreno, S; Fernández, G. Isolation and characterization of a dual function protein from Allium sativum bulbs which exhibits proteolytic and hemagglutinating activities. Plant Physiology and Biochemistry, v. 46, p. 403-13, 2008.

Pelosi, E; Lubelli, C; Polito, L; Barbieri, L; Bolognesi, A; Stirpe, F. Ribosome-inactivating proteins and other lectins from Adenia (Passifloraceae). Toxicon, v. 46, p. 658-63, 2005.

Petrossian, K.; Banner, L. R.; Oppenheimer, S. B. Lectin binding and effects in culture on human cancer and non-cancer cell lines: Examination of issues of interest in drug design strategies. Acta Histochemica, v. 109, n. 6, p. 491-500, 2007.

Peumans, W. J.; Rougé, P.; Van Damme, E. J. M. The tomato lectin consists of two homologous chitin-binding modules separated by an extensin-like linker. Biochemical Journal, v. 376, p. 717-724, 2003.

Peumans, W. J.; Van Damme, E. J. M. Lectins as plant defense proteins. Plant Physiology, v. 109, n. 2, p. 347-352, 1995.

Rameshwaram, NR; Nadimpalli, SK. An efficient method for the purification and quantification of a galactose-specific lectin from vegetative tissues of Dolichos lablab. Journal of Chromatography B, v. 861, p. 209–17, 2008

Ratanapo, S.; Ngamjunyaporn, W.; Chulavatnatol, M. Interaction of a mulberry leaf lectin with a phytopathogenic bacterium, P. syringae pv mori. Plant Science, v. 160, p. 739-744, 2001.

Regente, M.; Taveira, G. B.; Pinedo, M.; Elizalde, M. M.; Tcchi, A. J.; Diz, M. S. S.; Carvalho, A. O.; La Canal, L.; Gomes. V. M. A Sunflower lectin witc antifungal properties and putative medical micology applications. Current Microbiology, 2014.

Reynoso-Camacho, R; De Mejía, EG; Loarca-Piña, G. Purification and acute toxicity of a lectin extracted from tepary bean (Phaseolus acutifolius). Food and Chemical Toxicology, v. 41, p. 21-7, 2003.

Ripoll, C; Favery, B; Lecomte, P; Van Damme, E; Peumans, W; Abad, P; Jouanin, L. Evaluation of the ability of lectin from snowdrop (Galanthus nivalis) to protect plants against root-knot nematodes. Plant Science, v. 164, p. 517-23, 2003.

Roberts, G. A. F. - “Chitin Chemistry”, The Macmillan Press, London (1992). [2] Goosen, M. E. A – “Applications of chitin and chitosan”, Technomic Publishing Company, Lancaster (1996).

Rodrigues, JS; Santos-Magalhães, NS; Coelho, LCBB; Couvreur, P; Ponchel, G; Gref, R. Novel core(polyester)-shell(polysaccharide) nanoparticles: protein loading and surface modification with lectins. Journal of Controlled Release, v. 92, p. 103-12, 2003.

Rojo, M. A. et al. Isolation and characterization of a new D-galactose-binding lectin from Sambucus racemosa L. Protein and Peptide Letters, v. 10, n. 3, p. 287-293, 2003.

Rougé, P; Peumans, WJ; Barre, A; Van Damme, EJM. A structural basis for the difference in specificity between the two jacalin-related lectins from mulberry (Morus nigra) bark. Biochemical and Biophysical Research Communications, v. 304, p. 91-7, 2003.

Rüdiger, H.; Gabius, H.J. 2001. Plant lectins: occurrence, biochemistry, functions and applications. Glycoconjugate Journal, 18:589-613.

Ruy, F; Vercesi, A E; Kowaltowsk, A J. Inhibition of specific electron transport pathways leads to oxidative stress and decreased Candida albicans proliferation. Journal Bioenergetics Biomembranes, v. 38, p.129–135, 2006.

Samarakoon P, Soubani AO. Invasive pulmonary aspergillosis in patients with COPD: a report of five cases and systematic review of the literature. Chron Respir Dis. 2008;5(1):19-27.

Santi-Gadelha, T. . Purification and biological effects of Araucaria angustifolia (Araucariaceae) seed lectin. Biochemical and Biophysical Research Communications, v. 350, n. 4, p.1050-1055, 2006.

Santos ALS, Soares RMA. Candida guilliermondii isolated from HIV-infected human secretes a 50kDa serine proteinase that cleaves a broad spectrum of proteinaceous substrates. Fems 2005: 43(1):13–20.

Santos, A. F. S. et al. Detection of water soluble lectin and antioxidant component from Moringa oleifera seeds. Water Research, v. 39, p. 975–980, 2005.

Seshagirirao, K; Prasad, MNV. Purification and partial characterization of a lectin from Euphorbia neriifolia latex. Biochemistry and Molecular Biology International, v. 35, p. 1199- 204, 1995.

Sharon, N., Lis, H. How proteins bind carbohydrates: lessons from legume lectins. Journal of Agricultural and Food Chemistry, v. 50, p. 6586-6591, 2002.

Shoham S, Levitz SM. The immune response to fungal infections. British Journal of Hematology 129: 569-582, 2005.

Singha, B; Adhya, M; Chatterjee, BP. Multivalent II [β-d-Galp-(1→4)-β-d-GlcpNAc] and Tα [β-d-Galp-(1→3)-α-d-GalpNAc] specific Moraceae family plant lectin from the seeds of Ficus bengalensis fruits. Carbohydrate Research, v. 342, p. 1034-43, 2007.

Sitohy, M; Doheim, M; Badr, H. Isolation and characterization of a lectin with antifungal activity from Egyptian Pisum sativum seeds. Food Chemistry, v. 104, p. 971-9, 2007.
Sobel JD, Wiesenfeld HC, Martens M, Danna P, Hooton TM, Rompalo A, et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med 2004; 351(9): 876-83.

Soilleux, EJ; Coleman, N. Medicine in focus. Transplacental transmission of HIV: a potential role for HIV binding lectins. The International Journal of Biochemistry & Cell Biology, v. 35, p. 283-7, 2003.

Sorensen, GL; Husby, S; Holmskov, U. Surfactant protein A and surfactant protein D variation in pulmonary disease. Immunobiology, v. 212, p. 381-416, 2007.

Spilatro, SR; Cochran, GR; Walker, RE; Cablish, KL; Bittner, CC. Characterization of a new lectin of soybean vegetative tissues. Plant Physiology, v. 110, p. 825-34, 1996
Stilmark, H. Ueber Ricin, ein gifties Fermente aus dem Samen von Ricinus comm. And einigen Euphorbiaceen. Dorpant University. 1888.

Stirpe, F; Bolognesi, A; Bortolotti, M; Farini, V; Lubelli, C; Pelosi, E; Polito, L; Dozza, B; Strocchi, P; Chambery, A; Parente, A; Barbieri, L. Characterization of highly toxic type 2 ribosome-inactivating proteins from Adenia lanceolata and Adenia stenodactyla (Passifloraceae). Toxicon, v. 50, p. 94-105, 2007.
Sun, J. Purification and characterisation of a natural lectin from the serum of the shrimp Litopenaeus vannamei. Fish & Shellfish Immunology, v. 23, n. 2, p. 292-299, 2007.

Suseelan, K. N. et al. Purification and characterization of a lectin from wild sunflower (Helianthus tuberosus L.) tubers. Archives of Biochemistry and Biophysics, v. 407, p. 241-247, 2002

Suseelan, KN; Bhagwath, A; Pandey, R; Gopalakrishna, T. Characterization of Con C, a lectin from Canavalia cathartica Thouars seeds. Food Chemistry, v. 104, p. 528-35, 2007.

Syed, F. B. F., Joshi, B. N., Sivaraman, H., Khire, J. M., Khan, M. I. Purification and characterization of a cell-surface lectin (lectin II) from Agrobacterium radiobacter NCIM 2443. Biochemistry and Molecular Biology International, v. 47, n. 3, p. 361-367, 1999.

Takahashi, KG; Kuroda, T; Muroga, K. Purification and antibacterial characterization of a novel isoform of the Manila clam lectin (MCL-4) from the plasma of the Manila clam, Ruditapes philippinarum. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, v. 150, p. 45-52, 2008.

Thakur, A; Rana, M; Lakhanpal, TN; Ahmad, A; Khan, MI. Purification and characterization of lectin from fruiting body of Ganoderma lucidum: Lectin from Ganoderma lucidum. Biochimica et Biophysica Acta (BBA) - General Subjects, v. 1770, p. 1404-12, 2007.

Thöm, I; Schult-Kronefeld, O; Burkholder, I; Goern, M; Andritzky, B; Blonski, K; Kugler, C; Edler, L; Bokemeyer, C; Schumacher, U; Laack, E. Lectin histochemistry of metastatic adenocarcinomas of the lung. Lung Cancer 2007; v. 56, p. 391-7, 2007.

Trindade, M. B. Structural characterization of novel chitin-binding lectins from the genus Artocarpus and their antifungal activity. Biochimica et Biophysica Acta, v. 1764, p. 146- 152, 2006.

Uryu K, Chen X-H, Martinez D, Browne KD, Johnson VE, Graham DI, et al. Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. Exp Neurol 2007;208:185-92.
Van Damme, EJM; Barre, A; Bemer, V; Rougé, P; Van Leuven, F; Peumans, WJ. A lectin and a lectin-related protein are the two most prominent proteins in the bark of yellow wood (Cladrastis lutea). Plant Molecular Biology, v. 29, p. 579-98, 1995.

Ven, C.; Matser, A. M.; Berg, R. W. Inactivation of Soybean Trypsin Inhibitors and Lipoxygenase by High-Pressure Processing. Journal of Agricultural and Food Chemistry, v. 53, n. 4, p. 1087-1092, 2005.

Wallis, R. Interactions between mannose-binding lectin and MASPs during complement activation by the lectin pathway. Immunobiology, v. 212, p. 289-99, 2007.

Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2008;46(3):327-60.

Wälti, MA; Walser, PJ; Thore, S; Grünler, A; Bednar, M; Künzler, M; Aebi, M. Structural Basis for Chitotetraose Coordination by CGL3, a Novel Galectin-Related Protein from Coprinopsis cinerea. Journal of Molecular Biology, v. 379, p. 146-59, 2008.

Wang X, Su B, Perry G, Smith MA, Zhu X. Insights into amyloid-β-induced mitochondrial dysfunction in Alzheimer disease. Free Radical Bio Med 2007;43:1569-73.

Wang ZY, Jenkinson JM, Holcombe LJ, Soanes DM, Veneault-Fourrey C, Bhambra GK, Talbot NJ. (2005). «The molecular biology of appressorium turgor generation by the rice blast fungus Magnaporthe grisea». Biochemical Society Transactions. 33 (Pt 2): 384–88. doi:10.1042/BST0330384. PMID 15787612.

Wang, H. X.; Bunkers, G. J. Potent heterologous antifungal proteins from cheeseweed (Malva parviflora). Biochemical and Biophysical Research Communications, v. 279, p. 669- 673, 2000.

Wang, H. X.; Ng, T. B. Concurrent isolation of a Kunitz-type trypsin inhibitor with antifungal activity and a novel lectin from Pseudostellaria heterophylla roots. Biochemical and Biophysical Research Communications, v. 342, n. 1, p. 349-353, 2006.
Wang, H.X.; Ng, T. B. Purification of Castamollin, a novel antifungal protein from Chinese chestnuts. Protein Expression & Purification, v. 32, p. 44-51, 2003.

Wang, H.X.; Ng, T. B. Purification of Castamollin, a novel antifungal protein from Chinese chestnuts. Protein Expression & Purification, v. 32, p. 44-51, 2003

Wang, X.; Bunkers, G. J. Potent heterologous antifungal proteins from cheeseweed (Malva parviflora), 2000. Biochemical and Biophysical Research Communications, v. 279, p. 669 - 673.

Warren Levinson & Ernest Jawetz - Microbiologia Médica e Imunologia – 7º edição 2005 Artimed Editora S.A.

Watanabe, T., Matsuo, I., Maruyama, J.-i., Kitamoto, K. & Ito, Y. (2007). Identification and Characterization of an Intracellular Lectin, Calnexin, from Aspergillus oryzae Using N-Glycan-Conjugated Beads. Bioscience, Biotechnology, and Biochemistry, Vol. 71, No. 11, (November 2007), pp. 2688-2696, 0916-8451

Wititsuwannakul, R; Wititsuwannakul, D; Sakulborirug, C. A lectin from the bark of the rubber tree (Hevea brasiliensis). Phytochemistry, v. 47, p. 183-7, 1998.

Wroblewski, S. et al. Potencial of lectin-N-(2-hydroxypropyl) methacrylamide copolymerdrug conjugates for the tratament of pre-cancerous conditions. Lournal of Controlled Release, v. 74, p. 283-293, 2001.

Wu, J. H.. Carbohydrate recognition factors of the lectin domains present in the Ricinus communis toxic protein (ricin). Biochimie, V. 88, N. 2, P. 201-217, 2006.

Yamaguchi, M; Jimbo, M; Sakai, R; Muramoto, K; Kamiya, H. Purification and caracterization of Microcystis aeruginosa (freshwater cyanobacterium) lectin. Comparative Biochemistry and Physiology Part B, v. 119, p. 593-7, 1998.

Yamaguchi, M; Ogawa, T; Muramoto, K; Kamio, Y; Jimbo, M; Kamiya, H. Isolation and Characterization of a Mannan-Binding Lectin from the Freshwater Cyanobacterium (Blue- Green Algae) Microcystis viridis. Biochemical and Biophysical Research Communications, v. 265, p. 703-8, 1999

Yang, H; Luo, T; Li, F; Li, S; Xu, X. Purification and characterisation of a calcium-independent lectin (PjLec) from the haemolymph of the shrimp Penaeus japonicus Fish & Shellfish Immunology, v. 22, p. 88-97, 2007.

Yao, Q.; Wu, C.; Luo, P.; Xiang, X.; Liu, J.; Mou, L.; Bao, J. A new chitin-binding lectina 91 from rhizome of Setcreasea purpurea with antifungal, antiviral and apoptosis-inducing activities. Process Bochemistry. v. 45, p. 1477-1485, 2010.

YE X Y; NG, TB. A chitinase with antifungal activity from the mung bean. Protein expression and Purification, v. 40, p. 230-236, 2005.

Ye, XY; Ng, TB; Tsang, PWK; Wang, J. Isolation of a homodimeric lectin with antifungal and antiviral activities from red kidney bean (Phaseolus vulgaris) seeds. Journal of Protein Chemistry, v. 20, p. 367–75, 2001.

Zabriskie TM, Jackson MD. (2000). «Lysine biosynthesis and metabolism in fungi». Natural Product Reports. 17 (1): 85–97. doi:10.1039/a801345d. PMID 10714900.

Zheng, S; Li, C; Ng, TB; Wang, HX. A lectin with mitogenic activity from the edible wild mushroom Boletus edulis. Process Biochemistry, v. 42, p. 1620-4, 2007.